FREQUENCY OF RE-BEDDING WITH DAIRY MANURE SOLIDS

Results – June 2008

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Frequency of Re-bedding with Dairy Manure Solids (DMS)

Summary

Comparison of daily or weekly bedding with dairy manure solids was studied over a one month period in both the summer and the winter at two farms. Bacterial levels in the solids, as well as pre and post milk cultures, somatic cell count and mastitis incidence were analyzed. Only *E. coli* levels were different between the daily and weekly bedding strategies, occurring only in the summer and only at one farm. Milk cultures after a full month on daily or weekly bedded pens were more likely to be positive for cows in daily bedded pens, while SCC was more likely to be > 200,000 for cows and > 100,000 for heifers in weekly bedded pens. However, SCC was affected more by lactation number and stage of lactation than by bedding frequency. Mastitis incidence over the study period was low, and was not affected by frequency of bedding change. Bedding animals with DMS on a weekly basis does not appear to have an adverse effect on milk quality and mastitis.

Background

The use of dairy manure solids as bedding on dairy farms is being implemented by a number of NY farms, even with skepticism from the veterinary community and agricultural advisors. They are implementing this practice for economic and availability reasons, but are finding, in addition, that it enhances cow comfort. A number of issues are of concern to these farms, including the relationship of this practice to herd health and somatic cell count. In a related NYFVI, and NYS Energy Research & Development Authority (NYSERDA) funded project, Cornell Waste Management Institute worked with 6 farms to assess this and other questions. There are still a number of questions surrounding the best management practices to use to implement this bedding option such as bedding frequency.

Discussions with these 6 producers throughout the research as well as a discussion convened at the 2006 NYSERDA conference involving many producers, identified a specific research question. How frequently should the animals be re-bedded? “Common wisdom” says it should be done often, while a close reading of the research literature suggests that to be ill-advised from the point of view of pathogen re-growth (as well as being less economical). Pathogens in organic

bedding reach high levels within a day or two of being placed in stalls and re-bedding provides fresh organic materials that serve as food for the organisms, thus frequent re-bedding may not be advisable. The need to conduct this project has come directly from our work with dairy producers in NYS.

Research Design

Two farms (P and S) using DMS directly from the separator in deep beds participated in this study. Each farm assigned 2 pens of animals to the study. The cows in each pen on each farm were of approximately the same parity and stage of lactation and were kept in the same pen for a one month period. The cows assigned to this study on farm P were 1st lactation animals, while those on farm S were multiparous (greater than 1st lactation). One of the pens was bedded daily with fresh DMS, while the other was bedded every 7th day. Stalls in each pen were scraped and raked daily as per normal farm practices.

At the beginning of the 2nd week of bedding, samples of used and unused bedding were taken to be analyzed. Samples were analyzed at Quality Milk Production Services (QMPS), Cornell University, Ithaca, NY for bacterial content and % moisture, pH, density and particle size at Brookside Laboratories in New Knoxville, OH. Samples were taken on day 0, 1, 2, 5, 6 and 7 in the 2nd week, as well as in the 4th week (Table 1). This was done during two seasons July to August, 2006 (summer), and in January to February, 2007 (winter). A protocol and calendars were given to each of the farms participating in the study to ensure that the pens were bedded according to project guidelines, and can be found in Appendix A. Dairy Comp 305 files were accessed for individual cows in each of the pens over the 2 study periods to assess individual cow SCC and mastitis incidence.

Table 1: Schedule of bedding and sampling frequency

Week/Day	Daily Bedded Pens			Weekly Bedded Pens			Unused DMS Pile
	New Bedding	Sample DMS (prior to re-bedding)	Milk Sample	New Bedding	Sample DMS (prior to re-bedding)	Milk Sample	Sample DMS
Week 1	Wed, Thurs, Fri, Sat, Sun, Mon, Tues	None	Wed	Wed	None	Wed	None
Week 2	Wed, Thurs, Fri, Sat, Sun, Mon, Tues	Thurs, Fri, Mon, Tues, Wed	None	Wed	Thurs, Fri, Mon, Tues, Wed	None	Wed, Thurs, Fri, Mon, Tues
Week 3	Wed, Thurs, Fri, Sat, Sun, Mon, Tues	None	None	Wed	None	None	None
Week 4	Wed, Thurs, Fri, Sat, Sun, Mon, Tues	Thurs, Fri, Mon, Tues, Wed	None	Wed	Thurs, Fri, Mon, Tues, Wed	None	Wed, Thurs, Fri, Mon, Tues
Week 5	Resume normal bedding	None	Wed	Resume normal bedding	None	Wed	None

Quarter milk samples on each cow in each pen in the summer and on the first 50 cows in each pen as they walked into the parlor in the winter, were taken on the first day of the 2 bedding frequency schemes (daily or weekly re-bed) and analyzed for bacterial concentrations at QMPS. At the same time, bulk milk samples for each cow were taken and sent to Dairy One in Ithaca, NY for somatic cell count (SCC) analysis. These same samples were taken again at the end of the month after having been on the bedding frequency scheme for one full month.

Results – Bedding Bacteria and Properties

Statistical analysis

Bedding bacterial levels and physical properties were analyzed using the JMP statistical analysis package. Statistical analysis was performed using either analysis of variance (ANOVA) for multiple comparisons with Student's t-test, or with linear regression. The analysis was run on a natural log transformation of the bacterial counts, and actual values of all other variables to help normalize the data. All of the analyses were performed with bacterial counts calculated on a volume basis.

ANOVA analysis measures the mean value of a response variable (i.e. cfu/ml *Streptococcus*) for each predictor variable (i.e. Season) and compares it to the variation of the mean response within each predictor. If the between-variable variation is large and the within-variable variation is

small, a significant difference is concluded. ANOVA, in this case, would tell whether or not season (winter or summer) has a significant effect on the mean cfu/ml of *Streptococcus* in the bedding (response). The Student's t-test (used when there are only 2 choices for the predictor) compares them to find the difference. For example, the level of *Streptococcus* in bedding in the winter is significantly higher or lower than in the summer.

Linear regression differs from the ANOVA analysis in that it examines the relationship between the predictor (i.e. day of sampling) and response variable (i.e. cfu/ml *Streptococcus*). It does not treat each predictor variable as a distinct point (as in the ANOVA), but considers the trend and measures whether the change in the response variable as the predictor variable changes is different from zero (i.e. as the day of sampling increases, the amount of *Streptococcus* in the bedding increases, decreases or remains the same).

Linear regression produces an equation in the form of $y = mx + b$, where:

- y = the response variable
- m = the slope of the line (i.e. the amount by which the y-level changes)
- x = the predictor variable, and
- b = the y-intercept (i.e. the level of y at time 0).

An r^2 value is also generated, which indicates how well correlated the x variable (predictor) is with the y-variable (response). In the example above, it would tell how much of the variation in cfu/ml *Streptococcus* in the bedding is due to the day on which it was sampled, or the passage of time. R-square values closer to 1 are a better fit. Slopes can then be compared to see if they are different from each other. In the example above, if the amount of *Streptococcus* increased over time in both the pen that was bedded daily, and the pen that was bedded weekly, linear regression could tell whether or not the increase was the same or different between the 2 bedding frequency schemes.

Bacterial Counts in Bedding

QMPS analyzes bedding for the following bacteria that are considered mastitis pathogens:

- Contagious pathogens:

    - *Staphylococcus aureus*
    - *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, to a lesser extent also *Streptococcus uberis*.
    - Mycoplasmas
- Environmental pathogens:
    - *Streptococcus* species (other than the above)
    - *Staphylococcus* species (other than above)
    - *Enterococcus* species
    - Coliform bacteria (including: *Escherichia coli*, *Klebsiella* species, and *Enterobacter* species)
    - *Pseudomonas* species
    - *Proteus*
    - *Serratia* species
    - *Prototheca*
    - *Corynebacterium* species
    - Other gram negative and gram positive bacteria

ANOVA was run on the average amount of each individual bacteria in the bedding based on the predictor variables farm (P or S), season (summer or winter), bedding type (unused or used), and bedding frequency (daily or weekly). Predictor variables that did not show a significant difference were removed from the model until only significant variables remained. Table 2 shows the results. Three of the nine bacteria differed between farms. *Streptococcus* and gram positive bacteria were greater at farm S than at farm P, and *Enterobacter* was greater at farm P than at farm S. Total bacterial content of the bedding was greater at farm S than at farm P. Bacterial content in the winter was greater than in the summer for *Streptococcus*, *Proteus*, and both gram positive and negative bacteria, while the coliform bacteria (*E. coli*, *Klebsiella* and *Enterobacter*) were all greater in the summer than in the winter. All bacteria were greater in the used bedding than in the unused bedding except *Staphylococcus* which was greater in the unused than the used, and *Proteus* where there was no difference. For bedding frequency, only *E. coli* showed a difference. The amount of *E. coli* in the weekly bedded stalls was greater than that in the daily bedded stalls.

Table 2: Anova results for bacterial analysis of bedding

Bacteria	Farm	Season	Bedding Type	Bedding Frequency
Streptococcus spp	S > P	Winter > Summer	Used > Unused	Daily = Weekly
Staphylococcus spp	S = P	Summer = Winter	Unused > Used	Daily = Weekly
E. coli	S = P	Summer > Winter	Used > Unused	Weekly > Daily
Klebsiella	S = P	Summer > Winter	Used > Unused	Daily = Weekly
Enterobacter	P > S	Summer > Winter	Used > Unused	Daily = Weekly
Proteus	S = P	Winter > Summer	Used = Unused	Daily = Weekly
Gram negative bacteria	S = P	Winter > Summer	Used > Unused	Daily = Weekly
Gram positive bacteria	S > P	Winter > Summer	Used > Unused	Daily = Weekly
Corynebacterium spp	S = P	Summer > Winter	Used > Unused	Daily = Weekly

Since the average amount of *E. coli* in the weekly bedded used bedding (7.4 log cfu/ml) was significantly greater than the average amount of *E. coli* in the daily bedded used bedding (5.5 log cfu/ml), anova analysis was run on *E. coli* levels in the bedding by farm, season and type to see where the difference lies (Table 3). At farm P, the used bedding in the weekly bedded pens (average of all days, n=30) had significantly higher levels of *E. coli* than the unused bedding (n=10) in the summer, and at farm S, this difference occurred in the winter. In addition, *E. coli* levels in the unused bedding in the summer at farm S started at significantly higher levels, which may be why there was no difference between used and unused in the summer at farm S.

Table 3: Anova results for *E. coli* levels in the bedding when bedded either daily or weekly

Farm	P				S			
Season	Summer		Winter		Summer		Winter	
Frequency	Daily	Weekly	Daily	Weekly	Daily	Weekly	Daily	Weekly
Unused (n=10)	4.2^{a}	4.2^{a}	1.5^{a}	1.5^{a}	7.9^{a}	7.9^{a}	0.0^{a}	0.0^{a}
Used (n=30)	5.5^{a}	9.1^{b}	5.3^{a}	5.2^{a}	7.5^{a}	10.3^{a}	3.6^{a}	4.8^{b}

Values with different superscripts in each column are significantly different from each other

Linear regression was run for each bacterium to determine if there was a linear change in the amount of bacteria in the bedding over time. A predictor variable (Day) was used to indicate the

day of the week in which the samples were taken (0, 1, 2, 5, 6 or 7). For pens that were bedded daily, days 1, 2, 5, 6 and 7 all represented used bedding that was 1 day or 24 hours old. For pens that were bedded weekly, the age of the bedding is the number of the day (i.e. day 1 = 1 day old, while day 7 = 7 days old). For all pens, day 0 is unused bedding. Frequency of bedding, as well as Frequency*Day were added to the model to determine if there was a change over time based on whether the cows were bedded daily or weekly, and if so, were they different from each other? Because bacterial levels in the bedding were different in the summer and winter for all but one of the bacteria, and there were some differences in bacterial levels between farms, this analysis was run separately for summer and winter as well as for each farm. Table 4 shows the results of this linear regression. If the amount of bacteria changed significantly over time, there is an equation in the table indicating the amount by which it increased or decreased. If there was no significant change, it is indicated by "NS" (not significant). The column labeled "Diff?" indicates if the change over time was significantly different between daily and weekly bedding.

In the summer, bacterial levels in the bedding did not change over time at farm S. At farm P, *Streptococcus, E. coli, Klebsiella,* and gram positive bacteria all showed a significant change over time, but that change was only different between daily and weekly bedding for *E. coli.* Figure 1 shows the change in *E. coli* levels over time for both daily (red) and weekly (blue) bedding at farm P in the summer. While *E. coli* levels remained the same throughout the week in bedding that was refreshed daily, it increased by 1 log cfu/ml per day in bedding that was not refreshed. However, the r^2 value of 0.21 indicates that the fit is not very good. It is fairly obvious from the graph that there is large variation in *E. coli* levels between samples.

In the winter, bacterial levels of *Streptococcus, Staphylococcus,* and gram negative and positive bacteria showed a significant change over time at either one or both of the farms. However, there were no significant differences in these changes over time between daily or weekly bedded bedding. Since the age of bedding in the daily bedded pens is always 24 hours old regardless of the "day", the fact that changes over time were not different between daily and weekly (other than for *E. coli*) indicates that it is not the age of the bedding that is increasing bacterial content. It may be the amount of fresh fecal material being left in the stall or what is being tracked in from the alley.

Table 4: Linear regression results for bacteria (log cfu/ml) in bedding over time

		Summer			Winter		
Bacteria	Farm	Daily	Weekly	Diff?	Daily	Weekly	Diff?
Streptococcus spp	P	15.7+0.2/day	15.7+0.2/day	No	2.4-0.4/day	NS	No
	S	NS	NS	No	18.3+0.2/day	17.7+0.3/day	No
Staphylococcus spp	P	NS	NS	No	3.8-0.6/day	2.4-0.4/day	No
	S	NS	NS	No	NS	NS	No
E. coli	P	NS	4.7+1.0/day	Yes	NS	NS	No
	S	NS	NS	No	NS	NS	No
Klebsiella	P	10+0.7/day	10+0.6/day	No	NS	NS	No
	S	NS	NS	No	NS	NS	No
Enterobacter	P	NS	NS	No	NS	NS	No
	S	NS	NS	No	NS	NS	No
Proteus	P	NS	NS	No	NS	NS	No
	S	NS	NS	No	NS	NS	No
Gram negative bacteria	P	NS	NS	No	NS	NS	No
	S	NS	NS	No	14.2+0.5/day	13.5+0.5/day	No
Gram positive bacteria	P	12+0.2/day	NS	No	16.9+0.1/day	17.1+0.1/day	No
	S	NS	NS	No	17.2+0.3/day	17.1+0.2/day	No
Corynebacterium spp	P	NS	NS	No	NS	NS	No
	S	NS	NS	No	NS	NS	No

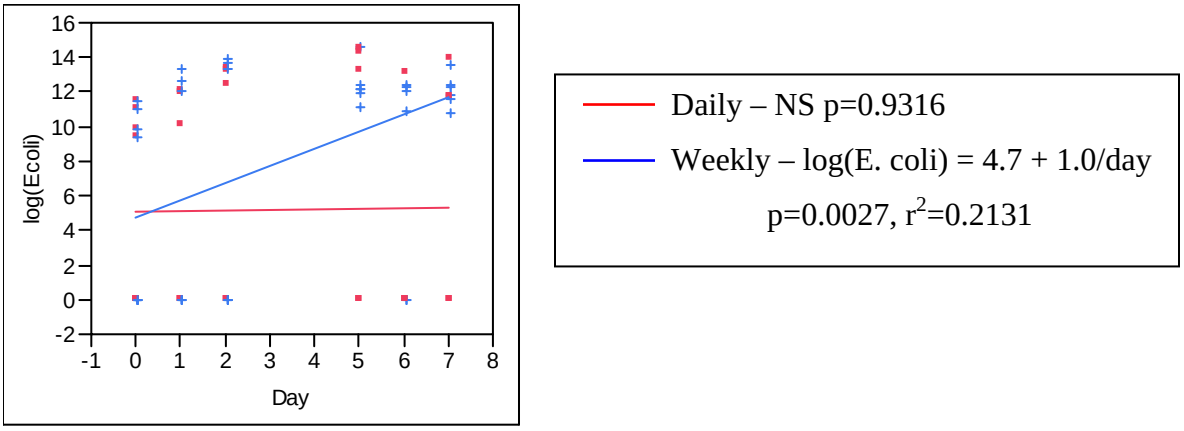

Figure 1: Linear regression results for *E. coli* (log cfu/ml) in bedding at farm P over time in the summer

Linear regression is somewhat misleading here, since the between sample variation is so large, and the fit of the line is not very good. Table 5 shows the level of *E. coli* in each of the individual

samples taken at farm P, as well as the mean for all samples on each day in the summer. Day 0 is unused bedding and was the same for both daily and weekly since it was taken from the DMS pile used to bed both pens. Samples 1 through 5 for Day 0 represent the samples taken in the 2nd week of the month on 8/1, 2, 3, 6 and 7, and samples 6 through 10 represent those taken in the last week on 8/15, 16, 17 and 21. For used bedding, samples 1 through 3 represent those taken in the second week of the month and samples 4 through 6 represent those taken in the last week of the month. For daily bedded bedding, the age of the used bedding is always 1 day or 24 hours old, while the age of the used bedding for the weekly bedded pen is the same as the day number. Because levels of *E. coli* vary so much between replicate samples, there is no difference in *E. coli* levels between any of the individual days regardless of whether they were bedded daily or weekly. In addition, comparison of each individual day between daily and weekly shows no difference, once again indicating that the age of the bedding has no effect on bacterial content.

Table 5: Levels of *E. coli* (log cfu/ml) in individual bedding samples at farm P over time in the summer

	Daily Bedded Bedding						Weekly Bedded Bedding					
Day of Study	0	1	2	5	6	7	0	1	2	5	6	7
Age	0	1	1	1	1	1	0	1	2	5	6	7
Sample 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0	0.0	11.6
Sample 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.3	10.9	11.9
Sample 3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.6	12.3	13.5
Sample 4	0.0	10.0	12.4	13.2	0.0	11.7	0.0	12.0	13.3	11.1	10.9	10.8
Sample 5	0.0	11.9	13.2	14.2	0.0	11.7	0.0	12.6	13.6	12.2	12.1	12.3
Sample 6	11.0	12.1	13.3	14.5	13.1	13.9	11.0	13.4	13.9	12.2	12.4	12.4
Sample 7	9.4						9.4					
Sample 8	9.8						9.8					
Sample 9	11.5						11.5					
Sample 10	0.0						0.0					
Average	4.2[a]	5.7[a]	6.5[a]	7.0[a]	2.2[a]	6.2[a]	4.2[a]	6.3[a]	6.8[a]	12.4[a]	7.9[a]	12.1[a]

Values with different superscripts in the last row are significantly different from each other

Physical Properties of Bedding

Bedding (both unused and used) was analyzed for % moisture, pH and particle size. It has been suggested in the literature that with more moisture and proper pH, bacterial populations thrive

(Appendix B: Literature Review). It has also been suggested that the amount of fine particles in the bedding has an effect on bacterial populations on the teat ends (the finer the material, the more likely it will stick to the teat ends, and therefore there will be a higher population of bacteria on the teat ends). This is hypothesized to, in turn, cause more mastitis by allowing entry of bacteria into the teats. Therefore, particle size was analyzed as % of particles < 2 mm (Fines1) and % of particles < 0.84 mm (Fines2).

Anova analysis was run on the average amount of moisture, pH and fine particles in the bedding based on the predictor variables farm (P or S), season (summer or winter), bedding type (unused or used), and bedding frequency (daily or weekly). Table 6 shows the results. Moisture was higher in the summer than in the winter which may be why there were greater levels of coliform bacteria in the bedding in the summer than in the winter. Moisture was also higher in the unused bedding than the used, and higher in the daily bedding than the weekly bedding. This makes sense since the bedding tends to dry out after it has been spread in the stall. The bedding in the weekly bedded stalls would have more time to dry out. The pH was different between farms and between bedding type, but not between season or between bedding frequency. Fine particles were greater at farm P, in the summer and in the used bedding. Only those less then 2 mm were different between bedding frequencies. Bedding in the weekly bedded pen would get matted and thus have less fine particles.

Table 6: Anova results for property analysis of bedding

Property	Farm	Season	Bedding Type	Bedding Frequency
Moisture	S > P	Summer > Winter	Unused > Used	Daily > Weekly
pH	P > S	Summer = Winter	Used > Unused	Daily = Weekly
Fines1	P > S	Summer > Winter	Used > Unused	Daily > Weekly
Fines2	P > S	Summer > Winter	Used > Unused	Daily = Weekly

Linear regression was run for each property to determine if there was a linear change in that property in the bedding over time in the same manner that it was run for bacteria. Table 7 shows the results of this linear regression. Moisture in the bedding decreased significantly over time at both farms during both the summer and the winter. The decrease in bedding moisture was

different between daily and weekly bedding only at farm S, in the summer, where weekly bedding decreased by 2.9% per day and daily bedding decreased by 1.2% per day. The only other difference in bedding properties between the daily and weekly bedding frequencies was at farm P where the percent of particles < 2 mm decreased by 1.7% per day in weekly bedding and increased by 2.3% per day in the daily bedding. Since increased moisture and increased fine particles have both been hypothesized to contribute to mastitis, it would be expected that the bedding in the daily bedded stalls would contribute more to mastitis than would the bedding in the weekly bedded stalls.

Table 7: Linear regression results for properties of bedding over time

		Summer			Winter		
Property	Farm	Daily	Weekly	Diff?	Daily	Weekly	Diff?
Moisture	P	67-2.1/day	66-3.1/day	No	72-0.6/day	72-1.0/day	No
	S	64-1.2/day	66-2.9/day	Yes	67-0.7/day	63-1.2/day	No
pH	P	8.9+0.1/day	8.9+0.1/day	No	NS	NS	No
	S	8.5+0.1/day	8.5+0.1/day	No	NS	NS	No
Fines1	P	76+1.2/day	75+0.9/day	No	58+2.3/day	56-1.7/day	Yes
	S	44+3.2/day	43+2.4/day	No	NS	NS	No
Fines2	P	28+4/day	27+4.6/day	No	14+1.5/day	NS	No
	S	18+1.7/day	16+2.5/day	No	18+1.1/day	19+1.4/day	No

Results – Milk Cultures, Somatic Cell Counts (SCC) and Mastitis Incidence

Statistical analysis

Milk culture results, SCC and mastitis incidence were analyzed using logistic and Poisson regression with the JMP statistical analysis package. Since Farm P had only heifers in the study pens, and Farm S had only multiparous cows, all analyses were run separately for each farm. Logistic regression measures the log odds of some response occurring based on a set of predictor variables. For example what are the log odds of getting a culture result of negative based on the pen in which the cow was housed. The results are given as a number that represents the log odds of the event. The anti-log of that number represents the actual odds of the event occurring.

Poisson regression is used when the outcome is a count, with large-count outcomes being rare events. For example, the number of mastitis events occurring based on the stage of lactation of the cows. Since the number of animals in the pens being studied at each farm and at each sampling differed, number of animals was used as an offset variable for these regressions. The offset variable transforms the model into a model of rates (i.e. number of mastitis events per number of cows) and helps to equalize the data between farms and samplings. The results are given as the difference in response between a specified level and the average of all other levels.

Milk Cultures

Culturing milk samples for mastitis pathogens can provide a great deal of valuable information for a dairyman. A single milk sample from an individual cow may provide significant information for that particular cow; however, multiple samples from many cows will provide much more information for mastitis prevention and control within the herd. Bacteria found in the milk of a cow can help identify infections early, facilitate treatment decisions and allow management changes that will have the greatest impact resulting in fewer new infections. Generally, reducing and/or preventing new infections will depend on appropriate milking procedures, cow (dry and milking) comfort and housing, heifer rearing and appropriate dry cow management.

QMPS analyzes milk for the same bacteria analyzed in the bedding. In this study, the milk culture results were divided into three categories: major pathogens (*Staph aureus*, *Strep* spp., *A. pyogenes*, serratia and proteus), minor pathogens (*Staph* spp., C. species, G+ bacillus) and negative culture results. Table 8 shows the initial culture results on the cows in each of the pens prior to implementation of the bedding frequency scheme. The number of animals with negative cultures was evenly distributed throughout the pens. Table 9 shows the culture results at the end of the bedding frequency scheme for those animals that had an initial culture result of negative.

Table 8: Initial culture results at Farm P and Farm S in each pen prior to implementation of bedding frequency scheme.

	Farm P				Farm S			
	Summer		Winter		Summer		Winter	
	Daily	Weekly	Daily	Weekly	Daily	Weekly	Daily	Weekly
Negative	58	54	21	26	35	36	14	15
Minor	10	21	5	2	5	7	0	3
Major	2	2	1	0	5	7	2	1
Total	70	77	27	28	45	50	16	19

Table 9: Post culture results at Farm P and Farm S in each pen after implementation of bedding frequency scheme for animals with initial culture negative.

	Farm P				Farm S			
	Summer		Winter		Summer		Winter	
	Daily	Weekly	Daily	Weekly	Daily	Weekly	Daily	Weekly
Negative	53	54	19	22	27	23	13	12
Minor	5	0	2	1	7	10	1	3
Major	0	0	0	3	1	3	0	0
Total	58	54	21	26	35	36	14	15

Culture results at the end of the bedding frequency scheme were analyzed using logistic regression for those animals that had an initial culture negative (first row on Table 8) to see if the odds of getting a major or minor culture result were different based on the farm, season, frequency of bedding, lactation and stage of lactation. Season, frequency of bedding and stage of lactation had no effect on the odds of having a minor or major culture result at the end of the bedding frequency scheme. Only farm and lactation category had a significant effect on culture results and only for the odds of having a minor versus a negative culture result. There were no variables that had an effect on having a major versus a negative culture result. The log odds of having a minor pathogen culture result versus a negative for farm P versus farm S were -0.82 which translates to $e^{-0.82} = 0.44$. This means that it is estimated that the odds of having a minor versus a negative culture result at farm P are 44% less than at farm S. The difference between the 2 farms can be explained in part, by the fact that all of the cows at farm P are heifers and all of

the cows at farm S are multiparous. The log odds of having a minor pathogen culture versus a negative culture for 1st lactation versus 2nd or greater were -0.96 which translates to $e^{-0.96} = 0.38$. This means that it is estimated that the odds of having a minor versus a negative culture result for 1st lactation cows is 38% less than for multiparous cows. The difference in the odds between farm and lactation means that there is something else going on at farm S than just lactation number causing greater odds of having a minor culture result.

Poisson regression was run on the number of positive post culture results (both minor and major) of those cows that had a pre culture result of negative. Since the farms were significantly different from each other, they were run separately. Number of positive cultures was used as the response variable, and season, frequency of bedding, and the bacteria and properties of the bedding that showed a difference between daily and weekly bedding (i.e. *E. coli*, moisture and fine particles < 2 mm) were used as the indicator variables. Since the number of animals with initial negative culture results differed for each sampling, the number of cows was used as an offset for the model.

At farm S, table 9 shows that in the summer, there were 8 out of 35 cows (22.9%) in the daily bedded pen and 13 out of 36 (36.1%) in the weekly bedded pen that had positive post cultures. In the winter, there was 1 out of 14 (7.1%) and 3 out of 15 (20%) in the daily and weekly bedded pens respectively. At farm S, none of the indicator variables (season, frequency, *E. coli*, moisture or particle size) had an effect on the number of cows with positive culture results at the end of the bedding frequency scheme.

At farm P, table 9 shows that in the summer, there were 5 out of 58 cows (8.6%) with positive post culture results, all of which were in the daily bedded pens, and in the winter, there were 2 out of 21 (9.5%) on daily bedding and 4 out of 26 (15.4%) on weekly bedding. Poisson regression results showed that frequency of bedding and the amount of *E. coli* in the used bedding had a significant effect on the number of cows that would be expected to have a positive post culture result after having a negative pre culture result, but not in the direction that would be thought.

Contrasting the effect that daily versus weekly bedding has on the number of cows with positive post culture results, Poisson regression estimates that the difference in log mean between daily and weekly bedding is 2.1. This corresponds to a ratio of $e^{2.1} = 8.2$ of daily versus weekly bedding. That means, on average, it is estimated that the number of animals in the daily bedded pen that will have a positive post culture is 820% or 7.2 times greater than the number of animals that will have a positive post culture in the weekly bedded pen. In addition, the amount of *E. coli* in the used bedding is negatively correlated with the number of positive post cultures in a group of cows with pre culture results that were negative, according to the equation: log(# positives) = intercept + (-2.8*log(Used*E. coli*) + log(number of cows). Therefore, if there was a group of 100 cows on used bedding with 500,000 cfu/ml *E. coli*:

Log(# positives) = 32.6 + (-2.8*log(500,000)) + log(100) = 32.6 + (-2.8*13.1) + 4.6 = 0.52
Positives = $e^{0.52}$ = 2 animals with a positive culture
If those same cows were on used bedding with 1,000,000 cfu/ml *E. coli*:
Log(# positives) = 32.6 + (-2.8*log(1,000,000)) + log(100) = 32.6 + (-2.8*13.8) + 4.6 = -1.4
Positives = $e^{-1.4}$ = 0 animals with a positive culture

These results are contrary with common thinking. It is thought that the more often the cow is bedded, and the lower the amounts of bacteria in the stalls, the less likely the animals are to get contaminated. These results show the opposite. They do, however, agree with the hypothesis that the finer the bedding (daily bedded stalls had significantly greater % of fine particles) the more likely that bacteria will enter the teat (cows in the daily bedded pen at farm P had significantly more positive cultures).

Somatic Cell Counts

Because mastitis is frequently sub-clinical, a number of tests have been developed for detecting mastitis. Most tests estimate the somatic cell count (SCC) of a milk sample. All milk contains white blood cells known as leucocytes which constitute the majority of somatic (derived from the body) cells. It has been generally accepted that the cell count for “normal” milk is nearly always less than 200,000 cells/ml for cows and 100,000 cells/ml for heifers. Higher counts are

considered abnormal and indicate probable infection. Individual cow SCC was obtained prior to implementing the bedding frequency scheme on each farm as well as at the end of the month in both the summer and the winter.

Table 10 shows the initial SCC results on the cows in each of the pens prior to implementation of the bedding frequency scheme (pre SCC). Table 11 shows the post bedding frequency scheme results (post SCC).

Table 10: Pre SCC results at Farm P and Farm S in each pen prior to implementation of bedding frequency scheme.

	Farm P				Farm S			
	Summer		Winter		Summer		Winter	
	Daily	Weekly	Daily	Weekly	Daily	Weekly	Daily	Weekly
Normal	60	42	62	70	55	50	68	66
Abnormal	44	58	36	29	30	31	27	26
Total	104	100	98	89	85	81	95	92

Table 11: Post SCC results at Farm P and Farm S in each pen after implementation of bedding frequency scheme.

	Farm P				Farm S			
	Summer		Winter		Summer		Winter	
	Daily	Weekly	Daily	Weekly	Daily	Weekly	Daily	Weekly
Normal	52	27	55	58	41	29	55	59
Abnormal	8	15	7	12	14	21	13	7
Total	60	42	62	70	55	50	68	66

SCC at the end of the bedding frequency scheme were analyzed using logistic regression for those animals that had a normal pre SCC (first row of Table 10) to see if the odds of getting an abnormal SCC count were different than getting a normal count based on the season, frequency of bedding and stage of lactation. Stage of lactation was divided into early (up to 60 days in milk), mid (61 to 200), late (201 to 300) and extended (> 300 days in milk), and was different at each farm. Farm P had 19 and 16% of animals in extended lactation in the summer and winter,

respectively, while farm S had only 3 and 2%. At farm P, all of the variables had an effect on SCC (Table 12).

Table 12: Odds ratios for abnormal versus normal milk (post SCC) for cows with normal pre SCC at farm P.

Indicator Variable	Level 1	Level 2	Odds Ratio	p-value
Season	Summer	Winter	2.1	0.0442
Frequency of bedding	Daily	Weekly	0.44	0.0273
DIM	Extended	Early	3.7	0.0085
	Extended	Mid	2.8	
	Extended	Late	4.1	

The odds of having an abnormal SCC versus a normal SCC for summer versus winter was 2.1. This means that it is estimated that the odds of getting an abnormal SCC after a normal SCC in the summer is 210% of, or 1.1 times greater than, in the winter. For daily versus weekly bedded animals it is estimated that the odds of getting an abnormal cell count were 44% less for heifers bedded daily than for heifers bedded weekly. For stage of lactation, the odds of having abnormal versus normal SCC for animals in extended lactation was 2.7, 1.8 and 3.1 times greater than if the animal was in early, mid or late lactation respectively.

At farm S, the only variable that had an effect on SCC was season. The odds of having an abnormal versus a normal SCC in the winter was 33% less than in the summer. Stage of lactation was not an issue at farm S since 95% of the animals in the summer and 81% in the winter were in mid or late lactation.

Poisson regression was run on the number of animals with abnormal cell count at the end of the month for those animals that had a normal pre SCC. The number of animals with an abnormal cell count was used as the response variable, and season, stage of lactation, frequency of bedding, milk production and the bacteria and properties of the bedding that showed a difference between daily and weekly bedding were used as the indicator variables. Since the number of animals with initial normal cell count differed for each sampling, number of cows was used as an offset for the model.

At farm S, Table 11 shows that in the summer, there were 14 out of 55 cows (25.5%) in the daily bedded pen and 21 out of 50 (42%) in the weekly bedded pen that had abnormal cell count at the end of the bedding frequency scheme. In the winter, there was 13 out of 68 (19.1%) and 7 out of 66 (10.6%) in the daily and weekly bedded pens respectively. At farm S, the only indicator variable that had an effect on SCC was the amount of *E. coli* in the used bedding. The number of animals with an abnormal cell count was positively correlated with the log cfu/ml *E. coli* according to the equation: Log(# animals w/abnormal cell count) = intercept + (0.8*log(cfu/ml *E. coli*)) + log(num cows). Therefore, if a group of 100 cows were on used bedding with 100,000 cfu/ml *E. coli* (the lowest average amount found at farm S):

Log(# animals) = -11.5 + (0.8*log(100,000)) + log(100) = -11.5 + (0.8*11.5) + 4.6 = 2.3
Animals with abnormal cell count = $e^{2.3}$ = 10 animals with abnormal cell count

If that same group of 100 cows were on used bedding with 700,000 cfu/ml *E. coli* (the highest average amount found at farm S):

Log(# animals) = -11.5 + (0.8*log(700,000)) + log(100) = -11.5 + (0.8*13.5) + 4.6 = 3.9
Animals with abnormal cell count = $e^{3.9}$ = 49 animals with abnormal cell count

At farm P, the only variable that had an effect on the number of animals with abnormal cell count was frequency of bedding. In the summer, there were 8 out of 59 (13.6%) with abnormal cell count in the daily bedded pen and 15 out of 42 (35.7%) in the weekly bedded pen. In the winter, there were 7 out of 62 (11.2%) and 12 out of 70 (17.1%) in the daily and weekly bedded pens, respectively (Table 11). Poisson regression results showed that frequency of bedding had a significant effect on the number of cows that would be expected to have an abnormal cell count after having a normal cell count. Contrasting the effect that daily versus weekly bedding had on the number of cows with abnormal cell count, Poisson regression estimates that the difference in log(mean) between daily and weekly bedding is -0.67. This corresponds to a ratio of $e^{-0.67}$ = 0.51 of daily versus weekly bedding. That means, on average, it is estimated that the number of

animals in the daily bedded pen that will have an abnormal cell count is 51% less than the number of animals that will have an abnormal cell count in the weekly bedded pen.

Between the two farms, those variables that are commonly considered to have a negative effect on milk quality did have that effect (more *E. coli* and lower frequency of bedding resulted in a greater number of animals with abnormal cell count). However, these effects were opposite of the response for milk cultures. Also, the milk culture response was only at one farm, while SCC response was split between two. In addition, at the farm where *E. coli* had an effect (farm S), there was no difference in the amount of *E. coli* in the used bedding between daily and weekly bedding schemes. Because the farms responded differently, it is more likely that other variables, such as milking parlor procedure and/or cleanliness of the animal, are playing a bigger part in the number of animals with abnormal cell count.

Mastitis

Mastitis incidence over the study period was fairly low. At Farm P, 5 out of 400 animals (1.3%) had clinical mastitis during the study period. There were 3 incidences in the summer (2 were in the daily bedded pen and the other was in the weekly bedded pen), and 2 cases of mastitis in the winter, both of which occurred in the daily pen. At Farm S, 12 out of 350 animals (3.4%) had clinical mastitis. Three occurred in the summer (2 in the daily bedded pen and 1 in the weekly), and 9 occurred in the winter (4 in the daily bedded pen and 5 in the weekly).

Mastitis events were analyzed using logistic regression to see if the odds of getting mastitis were affected by season, frequency of bedding, stage of lactation and whether or not the pre SCC was normal or abnormal. At farm P, none of the variables had an effect on the number of mastitis events. At farm S, the only variable that had an effect on mastitis was pre SCC (Table 13). The odds of getting mastitis were 2.5 times greater for animals with abnormal cell count than with normal cell count prior to implementation of the bedding frequency scheme.

Table 13: Odds ratio for getting mastitis versus not getting mastitis at Farm S.

Indicator Variable	Level 1	Level 2	Odds Ratio	p-value
Pre SCC	Abnormal	Normal	3.5	0.0292

Poisson regression of the number of mastitis events against the same indicator variables used for milk cultures and SCC was run for each farm separately. None of the indicator variables had a significant effect on the number of mastitis events during the study period.

Conclusions

The frequency with which stalls are bedded with DMS has very little to do with the amount of bacteria found in the used bedding. The only bacteria that was found in significantly greater amounts in weekly versus daily used bedding was *E. coli*, and it occurred only in the summer at one farm and only in the winter at the other. Season had much more effect on bacterial levels than did frequency of bedding. Summer showed higher levels of coliform bacteria and *Corynebacterium* species, while winter showed higher levels of *Streptococcus* species and gram negative and positive bacteria.

Frequency of bedding had an effect on the moisture content (drier in weekly bedded stalls) and % of fine particles (less in the weekly bedded stalls). Both of these characteristics of bedding have been attributed to contribute to increased SCC and mastitis (greater moisture and fine particles causing higher SCC and more mastitis). If this is the case, then weekly bedding of DMS would have a positive impact on SCC and mastitis.

The odds of having a positive milk culture at the end of the bedding frequency scheme were not affected by frequency of bedding. It was affected by the farm and lactation number. Since farm P had only heifers, and farm S had only multiparous cows on the study, the two variables are basically the same. Heifers were less likely to have a positive post culture than 2nd or greater lactation cows.

The number of animals with positive post cultures at farm P was affected by frequency of bedding and the amount of *E. coli* in the bedding. However, cows in the daily bedded pens were 7.2 times more likely to have a positive post culture than those in the weekly bedded pen and *E.*

coli was negatively correlated, meaning that the more *E. coli* found in the bedding, the fewer animals with positive cultures. Since daily bedded pens had more moisture and fine particles than weekly bedded pens, increased positive cultures makes sense, but higher bacterial levels causing fewer animals to have a positive culture is hard to explain. There were no indicator variables at the other farm that had an affect on the number of animals with positive post cultures.

The odds of having an abnormal versus a normal post SCC after a normal pre SCC were affected at one farm by season (summer more likely than winter), frequency of bedding (weekly > daily) and stage of lactation (animals > 300 DIM more likely than all others), but only by season at the other (winter more likely than summer). Since season is the only common variable among the two farms, it is more likely that other variables such as management and cow cleanliness are more important than bedding frequency.

The number of animals with abnormal post SCC was affected by frequency of bedding at one farm and the amount of *E. coli* in the used bedding at the other. Weekly bedded cows were more likely to have an abnormal post SCC than daily bedded cows at the farm where weekly bedded cows were less likely to have a positive post milk culture. If SCC has a direct relationship with the amount of bacteria in the milk, this does not make a lot of sense. At the other farm, the amount of *E. coli* in the used bedding was positively correlated with the number of animals with abnormal post SCC. Because the farms responded differently, it is more likely that other variables, such as milking parlor procedure and/or cleanliness of the animal, are playing a bigger part in the number of animals with abnormal cell count.

Mastitis events over the study period were few. The odds of a cow getting mastitis were significantly higher for those cows that had an abnormal pre SCC at one farm. None of the indicator variables had an effect on the odds of getting mastitis at the other farm. In addition, the number of mastitis events was not affected by any of the indicator variables. If these trends are real it would be helpful to farmers to know. Having looked at only 2 farms for bedding frequency, it would be prudent to extend the study and add a few more farms to get a more comprehensive picture.

Frequency of bedding appears to have little effect on milk quality and mastitis. Less frequent bedding may even have a positive impact by reducing the moisture and the amount of fine particles. Daily bedding of DMS can be time consuming and expensive and may not have any positive impact on bacterial levels or milk quality and mastitis.

APPENDIX A

FREQUENCY OF RE-BEDDING PROTOCOL

2 pens at each farm

- Try to have animals in both pens around the same age, and same stage of lactation.
- We would like to have the same animals remain in their assigned pens for full month, so they should not be close to dry-off.
- Please print daily milk production for cows in each pen from July 25 through Aug 22.

Bedding

- Clean DMS bedding in both pens on Wed, July 25, 2007.
- Change bedding daily in one pen through Aug 22, then you may resume your regular bedding procedure.
- In the other pen, you may scrape and rake, but do not re-bed until the following Wed (Aug 1), then re-bed on Aug 8 and Aug 15. Leave until Aug 22, then you may resume your regular bedding procedure.

Milk Samples

- QMPS will come on July 25 and Aug 22 to take quarter samples for milk culture and samples for SCC on all cows in each of the 2 pens above.

Bedding Samples

- CWMI will begin sampling bedding on Aug 1 (Wed). We will take a sample of the unused bedding.
- Aug 2, 3, 6 and 7, (Thurs, Fri, Mon, Tues) we will sample used bedding from both pens and unused bedding
- Aug 8 (Wed), we will sample used bedding only.
- We will repeat this procedure beginning again on Aug 15 (Wed) when we will sample unused bedding.
- Aug 16, 17, 20 and 21, (Thurs, Fri, Mon, Tues) we will sample used bedding from both pens and unused bedding
- Aug 22 we will sample used bedding only
- Therefore, after we have taken the final sample on Aug 22, you are free to resume your normal bedding activities.

We will repeat this schedule sometime in January of 2008.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
July	**16 Ellen will call/e-mail farms with reminder about study.**		25 – Clean DMS bedding in 2 pens QMPS milk cultures and bulk samples	26 – Re-bed the pen assigned to daily bedding	27 Re-bed the pen assigned to daily bedding	28 Re-bed the pen assigned to daily bedding
29 Re-bed the pen assigned to daily bedding	30 Re-bed the pen assigned to daily bedding	31 Re-bed the pen assigned to daily bedding	**Aug** 1 CWMI will take unused bedding samples Re-bed both pens	2 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	3 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	4 Re-bed the pen assigned to daily bedding
5 Re-bed the pen assigned to daily bedding	6 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	7 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	8 CWMI will take unused bedding samples Re-bed both pens	9 Re-bed the pen assigned to daily bedding	10 Re-bed the pen assigned to daily bedding	11 Re-bed the pen assigned to daily bedding
12 Re-bed the pen assigned to daily bedding	13 Re-bed the pen assigned to daily bedding	14 Re-bed the pen assigned to daily bedding	15 CWMI will take unused bedding samples Re-bed both pens	16 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	17 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	18 Re-bed the pen assigned to daily bedding
19 Re-bed the pen assigned to daily bedding	20 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	21 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	22 CWMI will take unused bedding samples QMPS milk cultures and bulk samples Resume normal bedding	23 End of study until January 2008		

			procedures			
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
January	**14 Mary will call/e-mail farms with reminder about study.**		23 – Clean DMS bedding in 2 pens CWMI will take milk cultures Farm S SCC at Farm P	24 – Re-bed the pen assigned to daily bedding CWMI will take milk cultures at Farm P	25 Re-bed the pen assigned to daily bedding SCC at Farm S	26 Re-bed the pen assigned to daily bedding
27 Re-bed the pen assigned to daily bedding	28 Re-bed the pen assigned to daily bedding	29 Re-bed the pen assigned to daily bedding	**30** CWMI will take unused bedding samples Re-bed both pens	31 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	Feb 1 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	2 Re-bed the pen assigned to daily bedding
3 Re-bed the pen assigned to daily bedding	4 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	5 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	6 CWMI will take used bedding samples Re-bed both pens	7 Re-bed the pen assigned to daily bedding	8 Re-bed the pen assigned to daily bedding	9 Re-bed the pen assigned to daily bedding
10 Re-bed the pen assigned to daily bedding	11 Re-bed the pen assigned to daily bedding	12 Re-bed the pen assigned to daily bedding	13 CWMI will take unused bedding samples Re-bed both pens	14 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	15 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily bedding	16 Re-bed the pen assigned to daily bedding
17 Re-bed the pen assigned to daily bedding	18 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily	19 CWMI will take used and unused bedding samples Re-bed the pen assigned to daily	20 CWMI will take used bedding samples Resume normal bedding procedures	21 End of study CWMI will take milk cultures both Farms	22 SCC at Farm S	

	bedding	bedding	SCC at Farm P			

APPENDIX B

LITERATURE REVIEW

Cornell Waste Management Institute

Using Manure Solids as Bedding

Literature Review

December 2006

This work is part of a larger research and outreach project on the use of manure solids for bedding in dairy barns. That project is supported in part by the New York State Energy Research and Development Authority (Project # 8823), the New York Farm Viability Institute, Cornell Cooperative Extension and the NYS College of Agriculture and Life Sciences.

Information on the project can be accessed at: **http://cwmi.css.cornell.edu/bedding.htm**.

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Table of Contents

Summary

This work seeks to address questions regarding the use of dried manure solids (DMS) as bedding for dairy cows, specifically the relationship of DMS bedding to herd health. The concentration of pathogens in bedding, on teat ends and their relationship to mastitis is discussed in this review of the literature. Caution is needed in reviewing data since concentration based on wet weight vs. dry weight vs. volume will be different. There can also be a seasonal effect on bacterial numbers.

There are two types of bedding, organic and inorganic. Organic bedding materials contain nutrients needed for bacterial growth, while inorganic bedding materials do not. However, once any type of bedding becomes soiled (with fecal matter and urine), pathogen growth can be supported. Inorganic bedding, such as sand, may start out with low pathogen concentrations. Some organic bedding materials start out with lower concentrations than others. However, research shows that within 24-48 hours of being in the stall, pathogen levels in all organic bedding materials rise to similar concentrations. The addition of lime to the stalls is not supported by the literature.

The desirable frequency with which fresh organic bedding is added to the stalls is unclear. While "common wisdom" suggests frequent rebedding, the research literature indicates that pathogen levels peak after a couple of days and may decline thereafter. This may be a result of bacteria having eaten up the available nutrients and that frequent rebedding provides a new source of food resulting in higher bacterial counts. More work is needed on this subject.

The literature shows inconsistency regarding the relationship of bacterial concentrations in bedding to the bacterial concentration on teat ends. Factors such as particle size may be more important than simply bacterial counts in the used bedding. The relationship of teat end counts to mastitis is unclear and is reviewed below.

Researchers have generally stated the rule of thumb that bedding materials should be kept below a maximum bacterial count of 10^6 colony forming units (cfu) per gram of bedding wet weight. This number appears to be based on one study where there were no new cases of coliform mastitis when bedding counts were at 10^4 and 10^5 one summer, but there were several new cases the following summer when bedding counts were at 10^7 cfu/g wet weight (Bramley and Neave, 1975). This paper does not claim that 10^6 colony forming units (cfu) per gram of bedding wet weight is a critical level and it represents data from only two summers on one farm. A few studies show a correlation between the number of bacteria in the bedding and/or the number on the teat ends and mastitis while a number of studies show no correlation. Few studies examined the relationship between bedding pathogens and milk quality.

Several studies have been conducted on the differences between herds that have low average SCC counts and herds that have high average SCC counts. Other studies look at the value of SCC count in determining intra-mammary infection (IMI) status in herds. High SCC is correlated with decreased milk production. SCC is measured both with a bulk tank sample (BTSCC) and with individual milk samples from each cow. BTSCC can be a good indicator of a herd's general udder health status, with high BTSCC generally indicating a problem with contagious mastitis. Herds with lower BTSCC have lower subclinical mastitis and better general udder health. However, the presence of leucocytes in the udder helps protect it from getting other mastitis, therefore low SCC (less than 20,000) appears to predispose cows to getting environmental mastitis. By looking at individual cow SCC over a period of several months, patterns can be established for each cow. Spikes in individual cow SCC usually indicate environmental mastitis and are often short in duration. When SCC is done on a monthly or other low frequency basis, these spikes may be missed. Thus typical BTSCC cannot generally be used to diagnose environmental mastitis at the herd level unless it is pervasive and persistent.

The impact of bedding, cleanliness of the udder and/or legs on the mastitis rate of a herd is unclear. Bedding may play a role in the cleanliness of the udder, and pre-milking udder hygiene may play a role in the amount of mastitis seen.

Other issues that may affect intramammary infection in dairy herds include stage of lactation and the dry period, parity (number of lactations), milking and milking machine factors including the use of post milking dips, teat end roughness and callosity, seasons of the year, nutrition, and housing conditions other than bedding.

Introduction

Dairy farms in NYS are under increasing pressure to improve their management of manure. Increasing environmental regulation and neighbor odor concerns are factors encouraging the separation of manure solids rather than direct spreading of manure. Implementation of anaerobic digestion on farms for energy recovery and for odor management also generates manure solids. Thus, the need for a use for the separated solids becomes ever more apparent.

Bedding is a costly and time consuming component of dairy farming that has implications for herd health as well as the environment and economics. The cost and availability of bedding fluctuates and good consistent bedding can be hard to find and expensive. Some bedding materials (i.e. straw and sawdust) result in additional nutrients being brought onto the farm, adding to nutrient management concerns.

In the northeast, there is increasing interest in and some limited experience with the use of dried manure solids, the semi-solid (25% solids) material derived from a manure stream run through a separator (DMS) for bedding. While interest is high, there is resistance on the part of some veterinarians, farm advisors, and farmers to using DMS as bedding primarily due to concerns that use of DMS will cause elevated levels of environmental pathogens that may negatively affect udder health (increased environmental mastitis) and milk quality.

The potential financial savings of using dried manure solids (DMS) are substantial and the potential to avoid bringing additional nutrients in bedding materials onto the farm is another benefit. Farmers using dried manure solids (DMS) report greater cow comfort than with other bedding materials they have used.

Mastitis is a costly disease to the dairy farmer. It is broken down into contagious mastitis (caused by bacteria that are found in the mammary gland and spread from cow to cow largely through the milking process), and environmental mastitis (caused by bacteria that live in the environment and spread through exposure to them in the environment). Control of contagious mastitis is sought through milking hygiene, the use of teat dips, treatment of infected animals in lactation, culling of animals with chronic infections, and dry cow anti-biotic therapy. Control of environmental mastitis is sought through stall and animal hygiene and through improvement of host resistance.

Because mastitis is frequently sub-clinical, a number of tests have been developed for detecting mastitis. Most tests estimate the somatic cell count (SCC) of a milk sample. All milk contains white blood cells known as leucocytes which constitute the majority of somatic (derived from the body) cells. It has been generally accepted that the cell count for “normal” milk is nearly always less than 200,000 cells/ml. Higher counts are considered abnormal and indicate probable infection. SCC can be done on individual cows or on bulk tank milk samples. Elevated SCC for environmental mastitis are often short-lived, so periodic SCC counts are less useful in evaluating environmental mastitis infections. High SCC has been associated with milk yield loss.

Low levels of leucocytes in the mammary gland may increase the incidence of infection by environmental pathogens such as coliforms. Herds that have effectively controlled contagious mastitis

pathogens (*Streptococcus agalactiae, Streptococcus dysgalactiae,* and *Staphylococcus aureus*) through programs of postmilking teat disinfection and dry-cow therapy, tend to have more problems with environmental mastitis pathogens.

The following bacteria are those commonly considered mastitis pathogens:

Contagious pathogens:

- *Staphylococcus aureus*
- *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, to a lesser extent also *S. uberis.*
- Mycoplasmas

Environmental pathogens:

- *Streptococcus* species (other than the above)
- *Enterococcus* species
- Coliform bacteria (including: *Escherichia coli, Klebsiella* species, and *Enterobacter* species)
- *Pseudomonas* species
- *Proteus*
- *Serratia* species
- *Prototheca*
- *Corynebacterium* species

The following is a summary of research literature on the contribution of bedding to cow health and milk quality and other issues pertaining to bedding material.

Bacterial Counts in Bedding

There are two types of bedding, organic and inorganic. Organic bedding materials contain nutrients needed for bacterial growth, while inorganic bedding materials do not. However, once any type of bedding becomes soiled (with fecal matter and urine), pathogen growth can be supported. Inorganic bedding, such as sand, may start out with very low pathogen concentrations. Some organic bedding materials, such as composted manure solids, start out with lower concentrations than others. However, research shows that within 24-48 hours of being in the stall, pathogen levels in all organic bedding materials rise to similar concentrations. Thus the expense of composting DMS prior to bedding may not accomplish a reduction in pathogen exposure. Similarly, the addition of lime to the stalls is not supported by the literature. There can also be a seasonal effect on bacterial numbers.

The desirable frequency with which fresh organic bedding is added to the stalls is unclear. While "common wisdom" suggests frequent rebedding, the research literature indicates that pathogen levels peak after a couple of days and may decline thereafter. This may be a result of bacteria having eaten up the available nutrients and that frequent rebedding provides a new source of food resulting in higher bacterial counts.

Calculating Concentrations

The numbers of bacteria found in bedding materials is reported on both a dry and wet weight ("as is") basis in the research literature which is confusing. One researcher has suggested reporting pathogen concentrations on a volume rather than a weight basis (Gabler, et al 2001). How the numbers are measured should be kept in mind when looking at data. When comparing bacterial counts within the same type of bedding material, it might makes sense to do it on a dry weight basis. For example, dry weights might be used when examining the change in concentrations over time in the same barn using the same

bedding. Comparing different materials with very different densities, such as sand and DMS, is challenging since the bedding in a stall of sand will weigh more than a stall with DMS. For the same volume of material, the higher density of sand would result in lower reported concentrations than a lighter material so the sand would "look cleaner." Knowing what is important in terms of what the cows are exposed to is unclear.

Wet vs. Dry Weight Calculations:

The number of bacteria can be reported as colonies per gram of material on an "as is" wet weight basis. In order to determine the concentration on a dry weight basis, the lab will dry the material after testing it for bacteria and convert the number of colonies to a dry weight basis.

Sample calculation to convert wet to dry weight bacterial concentrations
1000 colonies/ 100 grams wet weight
Sample is 20% solids, 80% moisture by weight
thus:
1000 colonies/20 grams solids

Weight vs. Volume Calculations:

The number of bacteria can be reported as colonies per gram of material on an "as is" wet weight basis. In order to determine the number of colonies per ml of material on an "as is" basis, the lab will need to weigh a known volume of the bedding. The number of colonies per ml can then be calculated on a volume basis as follows: (cfu/g wet weight) * (wet weight/volume).

Sample calculation to convert weight to volume bacterial concentrations
1,000,000 colonies/gram wet weight
100 milliliters of the bedding weighs 33 grams
thus:
(1,000,000 colonies/grams) * (33 grams/100 ml)

Comparison of Fecal Coliform Counts in Used and Unused DMS on One Farm Calculated on Wet (as is), Dry and Volume Basis

NOTE: These data are from one set of samples and are provided only as an example.

	Dry Matter (%)	Volume (g/ml)
Unused	37	0.41
Used	71	0.23

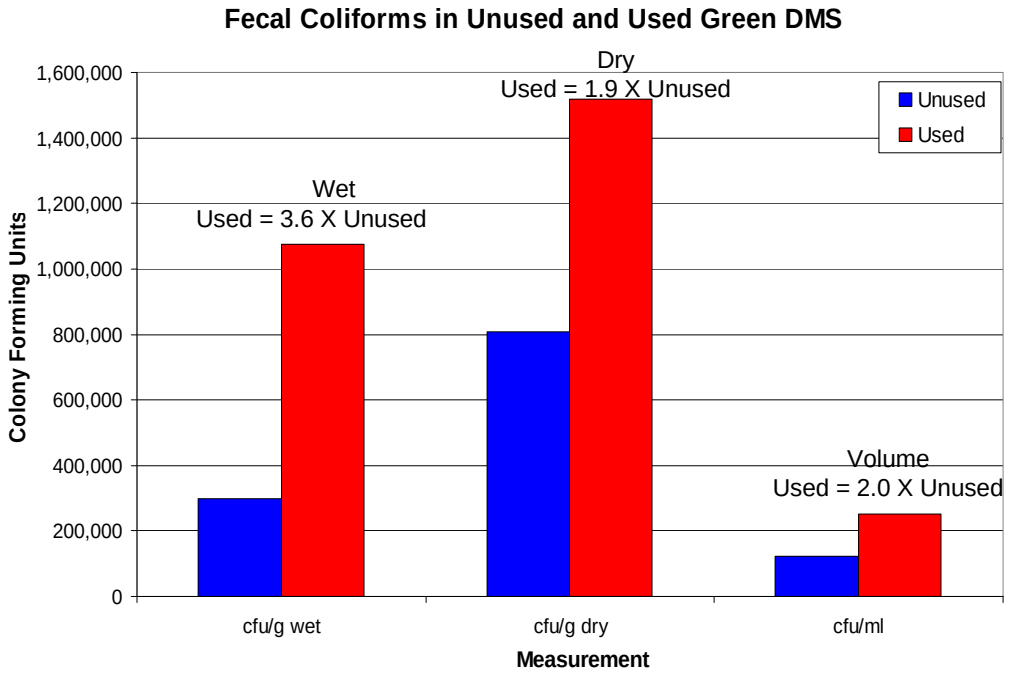

Figure 1.

Comparison of Fecal Coliform Counts in Different Bedding Materials Calculated on Wet (as is), Dry and Volume Basis

NOTE: These data are from one set of samples and are provided only as an example.

	Dry Matter (%)	Volume (g/ml)
Sand	96	1.16
CDMS	60	0.25
GDMS	66	0.32

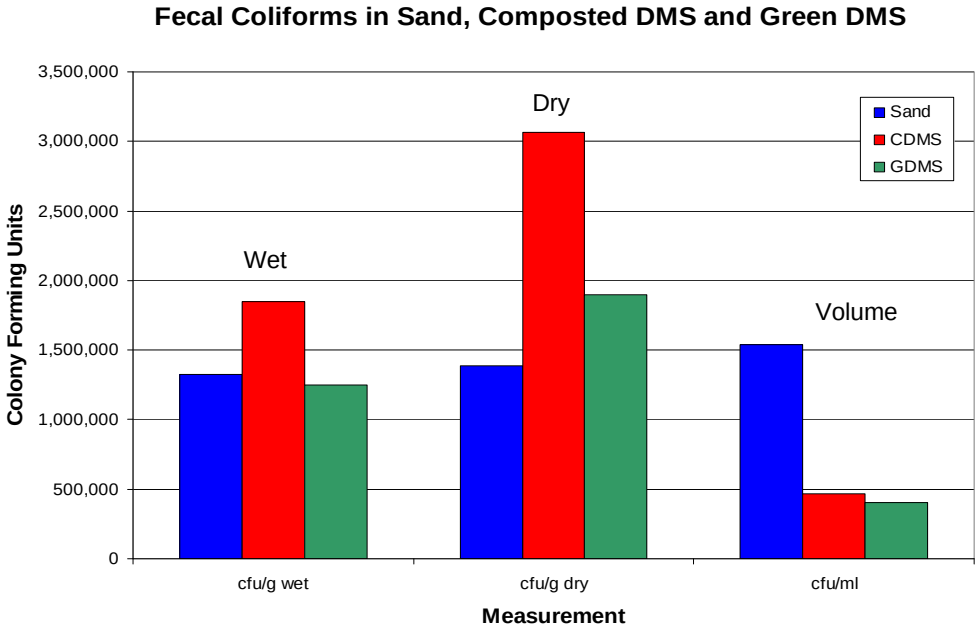

Figure 2.

Figures 1 and 2 show the difference between fecal coliform concentrations reported on a wet weight (as is), dry weight and volume basis. When comparing the same bedding source used vs. unused (Fig. 1), the fact that the material has dried in the barn so that the used is drier than the unused means that the difference between concentrations made on a wet weight basis is much greater than the difference on a dry weight basis or on a volume basis.

When comparing different materials, the impact of wet vs dry vs volume measures is more apparent. Fig. 2 shows that in one set of tests used, sand bedding was comparable to the green DMS and lower than composted DMS on a wet weight basis, but is much higher in fecal coliform when looked at on a volume basis. Note: These data are from one set of samples and are provided only as an example.

Organic vs. Inorganic Bedding Materials

Brim and Timms (1989) – wet weight basis

- Trial to evaluate growth of environmental mastitis pathogens (*E. coli, K. pneumoniae* and *S. uberis*) in various bedding materials (all materials were clean – never used in a barn)
- Inorganic bedding sources (sand, limestone, and limestone treated with pine disinfectant) showed rapid bacterial growth by 6 hours and significantly higher growth of all organisms in 6-54 hours as compared to oat straw and cedar sawdust.
- Organic bedding sources (oat straw and cedar sawdust) showed a bimodal growth curve with increased bacterial growth at 6-24 hrs (slower rate than inorganic), followed by a decline from 36-54 hours. By 96-120 hours, coliform organisms in the oat straw and cedar sawdust were similar or higher than inorganic bedding sources.
- Coliform numbers remained elevated at 96 hours, while strep numbers declined for all bedding materials.

Hogan, et al (1989a) – dry weight basis

- Independent comparison of bedding materials showed mean seasonal bacterial counts measured over one year of used organic materials (sawdust and chopped straw) had significantly higher gram-negative, coliform, *Klebsiella* species and streptococcal bacteria than used inorganic materials (sand and crushed limestone)

Janzen, et al (1982) – wet weight basis

- *E. coli*, Enterobacter and *Streptococcus* counts in used and unused crushed limestone bedding < than DMS = 50:50 mixture of limestone and DMS. ($P < 0.05$)
- *Staphylococcus aureus* and *Staph. epidermis* counts in crushed limestone < DMS = 50:50 mixture. ($P < 0.05$)

Kristula, et al (2005)

- Comparison of bacterial counts in clean sand (CS) and recycled sand (RS)
- There was a significant increase in bacterial counts from day 0 to d 1 for gram-negative bacteria, coliforms, and *Streptococcus* spp. in both winter and summer for both CS and RS.
- In the winter, counts of the above bacteria did not differ from days 1 – 7.
- In the summer, gram-negative counts did not differ from d 1-7, but coliform counts were lower on d1 than days 5-7 and *Klebsiella* spp. counts were lower on d 1 than on d 3-7.
- The number of *Streptococcus* spp was high in both CS and RS during the sampling periods.

LeJeune and Kauffman (2005) – volume basis

- Took used bedding from the stalls and brought them into the lab and inoculated with *E. coli* O157:H7. Samples were taken over a period of 112 days.
- *E. coli* O157:H7 survived at higher concentrations in used sawdust bedding than in sand.

Newman and Kowalski (1973) – wet weight basis

- Large numbers of *Klebsiella* were isolated from unused sawdust bedding and storage bins in a 54-cow dairy herd having trouble with *Klebsiella* mastitis.
- At the second collection, *Klebsiella* numbers decreased which coincided with a change in bedding from sawdust to sand.
- According to the authors, the role of sawdust as a possible source of *Klebsiella* organisms is not unequivocal in this report and requires additional study. In this context it should be emphasized that changes in bedding from sawdust to sand preceded the decrease in the number of *Klebsiella* isolates in the milk and that a high percentage of sawdust samples from varied sources did contain *Klebsiella* organisms.

Zdanowicz (2002) dry weight basis – (fresh bedding added every 7 days)

- Sand Bedding:

- Coliforms: d 0 < d 1 = d 2 = d 6
- *Klebsiella* species: d 0 < d 1 < d 2
  d 1 = d6, 2 = d 6
- *Strep.* species: d 0 < d 2
  d 1 < d 6

  d 1 = d 2, d6 = d2

- Sawdust Bedding:
  - Coliforms: d 0 < d 1 < d 2 = d 6
  - *Klebsiella* species: d 0 < d 1 < d 2 = d 6
  - *Strep.* species: d 0 < d 1 = d 2 < d 6

Zdanowicz, et al (2004) dry weight basis - (fresh bedding added every 7 days)

- Sand Bedding:
  - Coliforms: d 0 < d 1 = d 2 = d 6
  - *Klebsiella* species: d 0 < d 1 < d 2 = d 6
  - *Strep.* species: d 0 < d 1 < d 2 < d 6
- Sawdust Bedding:
  - Coliforms: d 0 < d 1 < d 2 = d 6
  - *Klebsiella* species: d 0 = d 1 < d 2 = d 6
  - *Strep.* species: d 0 < d 1 < d 2 = d 6

Fairchild (1982) – dry weight basis

- Average total coliform counts over 9 weeks in used bedding were higher in sawdust (4.1×10^6) and paper (8.7×10^4) than in sand ($< 1.0 \times 10^3$) and lime ($< 1.0 \times 10^3$). The same was true for *Klebsiella.*

Comparison of Organic Bedding Materials

Bramley and Neave (1975) – wet weight basis

- $10^4 – 10^5$ coliforms/g wet weight in all used bedding materials (sand cubicles, straw yards, wood shaving yards, sawdust yards) on one farm in 1971-72.
- 10^7 coliforms/g wet weight in used sawdust yards on the same farm in 1972-73.

Hogan, et al (1989a) – dry weight basis

- *Klebsiella:* used sawdust > straw
- Streptococcal counts: straw > sawdust.

Hogan, et al (1990) – dry weight basis

- Gram-negative, coliform and streptococcal counts: used chopped newspaper = used corn cobs
- Staphylococcal counts: used chopped newspaper < used corn cobs
- Gram-negative and staphylococcal counts: used chopped newspaper > used wood shavings
- Streptococcal and coliform counts: used chopped newspaper = used wood shavings

Rendos, et al (1975) - wet weight basis

- Bedding only replaced where manure scraped – sampling at 7, 14 and 21 days old
- Unused bedding – pooled means from 9 samples/week
  - Total coliforms: straw > sawdust = shavings
  - *Klebsiella*: sawdust > shavings = straw
  - *Strep.*: straw > sawdust = shavings
  - *Staph.*: straw = sawdust > shavings

- Unused vs used: all organisms significantly different
- Used bedding – pooled means from 9 samples/week
  - Total coliforms: no difference
  - *Klebsiella*: no difference
  - *Strep.*: straw > sawdust = shavings
  - *Staph.:* straw > sawdust > shavings
- Used bedding by week (bedding remained in the stalls over a 3 week period)
  - Total coliforms: no difference between weeks
  - *Klebsiella*: no difference between weeks
  - *Strep.*: wk 1 = wk 3 > wk 2
  - *Staph.*: wk 3 > wk 2, no difference between wk 1 and 2 or 1 and 3.

Zehner, et al (1986) dry weight basis - bacteria grown in bedding materials that were not exposed to urine or feces or in a barn environment at all – all samples were sterilized before inoculation.

- Growth of all bacteria: DMS > straw > hardwood chips > paper = sawdust
- In general, paper and softwood sawdust did not support growth of any of the bacteria (*E. coli, K. pneumoniae* and *S. uberis*.
- *Klebsiella* counts were significantly greater than *E. coli* counts in all bedding materials. Coliforms were significantly greater than *S. uberis* counts.
- The most rapid changes in growth of *Klebsiella* occurred in the first 24 h after inoculation with populations stabilizing after about 54 h.
- Coliforms grow more rapidly and decline less rapidly than environmental streptococci on all types of bedding studied.
- By comparing these results with data from studies under barn conditions, it appears that high bacterial counts under barn conditions are influenced by factors more complex than type of bedding used.

Composting and Addition of Lime and other Bacteriocides

Carroll and Jasper (1978) – wet weight basis

- Total coliforms directly from the separator were about 10^7/g wet weight at about 80% moisture.
- After composting for 9 months, they ranged from 0 to 10^4.
- Once they were used as free stall bedding for several months, they ranged from 10^6 to 10^8.

Mote, et al (1988) – wet weight basis

- Composting manure solids in static piles decreased the number of coliforms and gram-negative bacteria to below detectable numbers, but as composting continued over the 10-wk period, both coliforms and gram-negative bacteria increased in numbers to that of fresh DMS (coincided with decline in internal temperature of piles).
- No justification for composting before use.

Fairchild, et al (1982) – dry weight basis

- *Klebsiella*: unused sawdust = unused sawdust plus lime.
- There was a significant difference between unused and used, but no significant increase after 1st week, with a reduction from wk 1 to wk 3. (the stalls were re-bedded after 1 week for 3 weeks)

Ward, et al (2002) – wet weight basis

- Studied 4 dairy farms that used straw yards for bedding
- The pH of the top layers of straw was usually between 8.5 and 9.5
- Adding lime daily to the top layer of the straw failed to raise the pH to levels at which *Escherichia coli* and *Streptococcus uberis* do not survive.

- Most of the counts of *E. coli* and fecal streptococci in the top layers of straw were above 10^6 colony-forming units/g.

Hogan & Smith (1997) – looked at bacteria counts in sawdust only (control), sawdust plus lime (treatment 1) and sawdust rebedded daily (treatment 2)– dry weight study

- Treatment effects on bacterial numbers and pH were limited after 1 day in the stall. The ability of lime to alter bacteria counts and pH apparently was diminished within 48 hours after application.
- Day 1: All bacteria: treatment 1 < treatment 2 = control
- Day 2: *Klebsiella* species: treatment 1 < treatment 2; treatment 1 = control
- Control: *Strep.* species, *Klebsiella* species, dry matter, pH: d 1 = d 2 = d 6
  Gram-negative, Coliforms: d 1 > d 6
- Treatment 1: All bacteria: d 1 > d 2 = d 6
- Treatment 2: All bacteria: d 1 = d 2 = d 6

Hogan, et al (1999) – additives to DMS and sawdust to reduce counts – dry weight basis

- Recycled manure – Gram negative counts
  - Unused: DMS > all treatments (DMS + lime = DMSL; DMS + acidic conditioner = DMSAcid; and DMS + alkaline conditioner = DMSAlk)
  - Day 1: DMS > DMSL; no other differences
  - Day 2 and 6: No difference in counts for any treatment.
- Recycled manure – Coliform counts
  - Unused: DMS > all treatments
  - Day 2: DMS = DMSAcid > DMSAlk
  - Day 1 and 6: No difference in counts for any treatment.
- Recycled manure – *Klebsiella* counts
  - Unused: DMS > all treatments
  - Day 1: DMS = DMSAcid > DMSL = DMSAlk
  - Day 2: DMS = DMSAcid > DMSL > DMSAlk
  - Day 6: No difference in counts for any treatment.
- Recycled manure – Streptococcal counts
  - Unused: DMS > all treatments
  - Day 1: DMS > all treatments
  - Day 2: DMS = DMSL = DMSAcid > DMSAlk
  - Day 6: No difference in counts for any treatment.
- Sawdust – Gram negative counts
  - Unused: SAW > all treatments (sawdust + lime = SAWL; sawdust + acidic conditioner = SAWAcid; sawdust + alkaline conditioner = SAWAlk)
  - Day 2: SAW > SAWAcid
  - Day 1 and 6: No difference in counts for any treatment.
- Sawdust – Coliform counts
  - No effect on counts with use of any of the additives at any time.
- Sawdust – *Klebsiella* counts
  - Unused: No difference in counts for any treatment.
  - Day 2: SAW > SAWAcid
  - Day 1 and 6: No difference in counts for any treatment.
- Sawdust – Streptococcal counts
  - Unused: SAW > SAWAcid
  - Day 2: SAW = SAWL = SAWAlk > SAWAcid
  - Day 1 and 6: No difference in counts for any treatment.

Seasons and Bacterial Counts in Bedding

Hogan, et al (1989a) – dry weight basis

- Bacterial counts in long straw differed among seasons of the year:
  - Gram-negative: summer = fall > winter = spring
  - Coliforms: summer > winter
  - *Klebsiella* species: no seasonal differences
- *Klebsiella* counts in sawdust: summer = fall > winter = spring

Smith, et al, (1985a) – wet weight basis [Note: Since concentrations were based on wet weight measures, the drier DMS in summer would show higher counts than the same material when wetter.]

- Highly significant effect of season on colony forming units (log_{10}) of coliforms in recycled manure used in free stalls. Colony forming units in used DMS were higher in summer compared with other seasons. Summer > fall > spring = winter.
- The same was true for the pelleted corn cob bedding used in maternity units. Highest cfu coliforms in summer and lowest in winter.
- No data on streptococcal numbers.

Todhunter, et al (1995) – dry weight basis

- The number of streptococci in bedding materials exceeded 10^6 cfu/g of dry weight for all bedding types during all seasons of the year.
- Streptococcal numbers in bedding of pelleted corn cobs were similar across seasons of the year.
- Season of the year had no effect on numbers of streptococci in bedding of wood shavings.
- The number of streptococci in recycled manure was lower ($P < .05$) during the summer than during the winter and spring.

Bacteria in Bedding and on Teat Ends

The literature shows inconsistency regarding the relationship of bacterial concentrations in bedding to the bacterial concentration on teat ends. Factors such as particle size may be more important than simply bacterial counts in the used bedding. The relationship of teat end counts to mastitis is unclear and is reviewed below.

Studies Showing Counts in Bedding Correlated with Counts on Teat Ends

Bishop, et al (1981)

- There was a significant difference in *E. coli* and *Enterobacter* counts between composted DMS (higher) and rubber mats and a significant difference on the teat ends (higher on cows bedded on DMS).

Fairchild (1982)

- *Klebsiella* teat end swabs and bedding samples were highly correlated (more on teat ends of cows bedded with sawdust than those bedded on lime).

Hogan and Smith (1997)

- Bacterial counts in bedding positively correlated with teat skin swabs.

Hogan, et al (1999)

- Recycled Manure: Coliforms
  - Day 2: Teat ends: DMS > DMSAlk

Bedding: DMS > DMSAlk

- Day 1 & 6: Teat ends: No difference
  Bedding: No difference

- Recycled Manure: *Klebsiella*
  - Day 2: Teat ends: DMS = DMSAcid > DMSL > DMSAlk
    Bedding: DMS = DMSAcid > DMSL > DMSAlk
  - Day 6: Teat ends: No difference
    Bedding: No difference

Janzen, et al (1982)

- *E. coli*, *Enterobacter* and *Strep.* spp. counts on teat ends were significantly less in cows bedded on crushed limestone vs. DMS or 50:50 mixture.
- *Staph. aureus* and *Staph. epidermis* counts on teat ends were significantly less in cows bedded on crushed limestone vs. DMS or 50:50 mixture.

Natzke and LeClair (1975)

- Large numbers of coliform bacteria were found on teat ends of cows bedded with sawdust artificially contaminated with coliform bacteria as compared to controls (sawdust not contaminated with coliform bacteria).

Zdanowicz (2002)

- There was a significant correlation between the mean "cow-bedding count 1" (time spent lying in a stall multiplied by the bacterial count for the stall) and the bacterial counts on teat swabs for cows housed on sand for coliforms and *Klebsiella* spp.
- There was a significant correlation between the mean "cow-bedding count 1" and the bacterial counts on teat swabs for cows housed on sawdust for coliforms, *Klebsiella* spp. and *Streptococcus* spp.

Zdanowicz, et al (2004)

- There were 2 times more coliforms and 6 times more *Klebsiella* bacteria on teat ends of cows housed on sawdust compared with those housed on sand.
- There were 10 times more *Strep*. spp. bacteria on teat ends of cows when housed on sand compared with sawdust.

Studies Showing Counts in Bedding Not Correlated with Counts on Teat Ends

Hogan, et al (1990) There is a positive correlation when data for all bacteria from each bedding type is pooled, but not necessarily each bacteria separately.

- Correlations between bedding counts and teat skin counts were not significant within bedding type.
- All bacteria: Teat Ends: week 1 > week 2 = week 3
  Bedding: week 1 = week 2 = week 3
- Gram-negative, coliform and *Klebsiella*: Teat ends: chopped newspaper = corn cobs
  Bedding: chopped newspaper = corn cobs
- Gram-negative: Teat ends: newspaper = wood shavings
  Bedding: newspaper > wood shavings
- *Strep.* spp.: Teat ends: newspaper > wood shavings
  Bedding: newspaper = wood shavings

- Appeared that adherence of bedding (due to particle size) had more to do with the difference in teat swab counts than the amount of bacteria in the bedding. (i.e. teat swab counts for gram-negative, coliform and *Klebsiella* differed between cows bedded on newspaper and corn cobs, but the amount of bacteria in the bedding didn't – corn cobs adhered more to the teats because of fine particle size and those cows had higher teat swab counts).

Hogan, et al (1999) – There is a positive correlation when data for all bacteria from each bedding type is pooled, but not necessarily each bacteria separately.

- Recycled Manure: Gram-negative
  - Day 1: Teat ends: DMS = DMSL > DMSAlk = DMSAcid
Bedding: DMS > DMSL and DMS = DMSAlk = DMSAcid
  - Day 2: Teat ends: DMSL > DMSAlk
Bedding: DMSL = DMSAlk
- Recycled Manure: *Strep.* species
  - Day 1: Teat ends: DMS > DMSAcid only
Bedding: DMS > DMSL = DMSAlk = DMSAcid
  - Day 2: Teat ends: DMS > DMSAcid only
Bedding: DMS = DMSL = DMSAcid > DMSAlk
- Recycled Manure: *Klebsiella*
  - Day 1: Teat ends: DMS = DMSL > DMSAcid = DMSAlk
Bedding: DMS = DMSL > DMSAcid = DMSAlk
- Sawdust – None of the bacterial counts on teat ends correlated with those in the bedding.

Rendos, et al (1975)

- Total Coliform counts on teats in sawdust > shavings = straw. There were no differences in coliform counts in the different bedding materials.
- *Klebsiella* counts on teats in sawdust > shavings > straw. There were no differences in bedding counts.
- *Strep.* spp. counts on teats in straw > shavings > sawdust. In bedding, straw > sawdust = shavings.
- *Staph.* spp. counts on teats in straw = sawdust > shavings. In bedding, straw > sawdust > shavings.
- Teat swab means between groups of cows (3 different sets in this trial) were significantly different from each other for all bacteria, indicating a cow effect on teat end contamination.

Zdanowicz (2002)

- There was no significant correlation for "cow-bedding counts 1" and teat end streptococci counts for cows bedded on sand.

Relationship of Bacteria in Bedding and on Teat Ends to Mastitis and Milk Quality

Researchers have generally stated the rule of thumb that bedding materials should be kept below a maximum bacterial count of 10^6 colony forming units (cfu) per gram of bedding wet weight. This number appears to be based on one study where there were no new cases of coliform mastitis when bedding counts were at 10^4 and 10^5 one summer, but there were several new cases the following summer when bedding counts were at 10^7 cfu/g wet weight (Bramley and Neave, 1975). This paper does not claim that 10^6 colony forming units (cfu) per gram of bedding wet weight is a critical level and it represents data

from only two summers on one farm. A few studies show a correlation between the number of bacteria in the bedding and/or the number on the teat ends and mastitis while a number of studies show no correlation. Few studies examined the relationship between bedding pathogens and milk quality.

Counts in Bedding and Mastitis

Bramley (1982)

- Large numbers of *Strep. uberis* were isolated from samples of straw bedding for cattle from farms which suffered a high incidence of *S. uberis* mastitis, but the results did not demonstrate a direct relationship between exposure to *S. uberis* from straw bedding and udder disease.

Fairchild (1982)

- Coliform counts > 10^6 in sawdust, but no new infections
- Unable to demonstrate a direct relationship between bacterial counts in bedding and rates of coliform or environmental IMI.
- High populations of coliforms will not necessarily cause infection under good management conditions.
- Type of bedding may be just one link in a chain of possible situations that promote mastitis.

Hogan, et al (1989a)

- Neither percentages of quarters infected at calving nor mean rates of clinical mastitis during the first 7 days of lactation were correlated with long straw bacterial counts (maternity area bedding).
- Linear relationships were significant among total rates of clinical mastitis during lactation and counts of gram-negative bacteria and Klebsiella species in lactating cow bedding.

Hutton, et al (1990)

- Prevalence of cows' environmental pathogen IMI was similar between high and low SCC herds as was the number of environmental organisms in bedding materials.

Todhunter, et al (1995)

- In recycled manure bedding, no correlation existed between the rate of environmental streptococcal IMI during the dry period and streptococcal numbers in bedding by season of the year.

Munoz, et al (2006)

- In a 5-mo study in a NY dairy herd performed during the summer of 2005, all of 9 samples of unused sand bedding tested negative for *Klebsiella.*
- 14 of 18 samples of used sand bedding contained *Klebsiella* at a median level of $10^{4.6}$ cfu/g
- It is hypothesized that fecal shedding of *Klebsiella* by dairy cows contributes to the presence of *Klebsiella* in the environment regardless of bedding type.

Counts on Teat Ends and Mastitis

Hogan, et al, (1990)

- IMI status of the quarters had no effect on teat swab counts

Neave and Oliver (1962)

- If teats are experimentally contaminated (> 30,000 colonies) with *Staph. aureus* (contagious mastitis pathogen) at the end of lactation, the quarters are much more likely to become infected than if the teats are lightly contaminated (30,000 colonies or less).
- The association of large numbers (15×10^6) of *Staph. aureus* at the apex and infection of the quarter was highly significant ($P < 0.001$) ($15 \times 10^6 > 30{,}000 = 60 =$ none).

- *Strep. uberis* was not recovered from either teats or orifices at the end of lactation, but was present in large numbers in six orifices 21 days later. All of these were associated with infected quarters. As *Strep uberis* was not applied to the teats at drying-off, it was assumed that those udders found to harbor it became contaminated from the environment of the dry cow.

Natzke and LeClaire (1975)

- No new coliform IMI despite large numbers on teat ends

Counts in Bedding Correlated with Counts in Milk

Hogan, et al (1988) (dry weight study).

- Gram-negative, coliform, and streptococcal counts in bulk tank milk were associated with bacterial counts in bedding materials
- Significant correlations among bacterial counts in bulk tank milk and bacterial counts in bedding were: gram-negative and gram-negative, coliform and coliform, coliform and *Klebsiella* species, and streptococcal and streptococcal.

Counts on Teat Ends Correlated with Counts in Milk

Janzen, et al (1982)

- *E. coli, Enterobacter* and *Strep.* spp. counts on teat ends and in the milk were significantly less in cows bedded on crushed limestone than in DMS or 50:50 mixture
- *S. aureus* counts on teat ends and in the milk were less in crushed limestone than DMS or 50:50 mixture.

Hygiene and Mastitis

The impact of bedding, cleanliness of the udder and/or legs on the mastitis rate of a herd is unclear. Bedding may play a role in the cleanliness of the udder, and pre-milking udder hygiene may play a role in the amount of mastitis seen.

Housing Hygiene and Mastitis

Barrett, et al (2005)

- Herds with prolonged periods on straw bedding in yards (exposed to rain, cleaned less frequently) were more likely to acquire environmental mastitis (12 herds in Ireland).

Bartlett, et al (1992)

- General sanitation in lactating cow housing was an important disease determinant of both coliforms and environmental streptococci.
- Improving general sanitation by 1 unit (scores of 1 – above average, 2 – approximately average and 3 – worse than average) was associated with a 57% reduction in the prevalence of coliform infection.

Howell, (1972)

- Survey of 50 herds in England having trouble with environmental mastitis (comparison of management)

- Cause of *E. coli* infection is believed to be the feces and infection is due to gross fecal contamination of the teat orifice. *E. coli* mastitis was rare in summer when cattle are pastured and only occurred in herds where zero grazing was practiced or where cows were kept for long periods in dirty yards during milking. Where *E. coli* occurred in cubicle herds, it was when there were obvious faults of the cubicles (i.e. wrong length, so dung fell in cubicle rather than alleyway and cows lay in it).

Peeler, et al (2000)

- Survey of management practices of British dairy herds with low somatic cell count (average 76,000 cells/ml) showed the following bedding variables lead to increased rate of clinical mastitis: straw in milking cow accommodations and mucking out the calving area less than once/month.
- The following bedding variables were shown to decrease the rate of clinical mastitis: cleaning out dry cow accommodation at least once/week, sawdust/wood shavings in the calving area and sawdust/wood shavings in dry cow accommodations.

Ward, et al (2002)

- Looked at 4 dairy farms that used straw for bedding.
- The farm with the lowest incidence of mastitis had the cleanest cows and the most satisfactory beds.
- Counts of E. coli and S. uberis were much higher in the beds of early lactation cows than in those of dry cows. Many of the early lactation cows were heavily and persistently contaminated with feces. Dry cows were much cleaner.

Barkema, et al (1999b)

- *E. coli* incidence higher if lactating cows are not allowed to graze at night.
- *S. aureus* and *S. dysgalactiae* incidence lower with thicker layer of straw in calving pen
- *S. dysgalactiae* incidence lower with thicker layer of straw in cubicles of dry cows
- *S. uberis* incidence higher with disinfection of cubicles of lactating cows.

Schukken, et al (1991)

- *E. coli* mastitis incidence lower if cubicles cleaned of manure, and with rubber mats at calving site, higher with complete cleaning of dry cow cubicles.
- *S. aureus* incidence lower with higher amount of bedding in cubicles.

Elbers, et al (1998)

- The following risk factors were associated with a higher rate of clinical mastitis caused by *E. coli*: no disinfection of the maternity area after calving, use of a thick layer of bedding in the stall.
- The following risk factors were associated with a higher rate of clinical mastitis caused by *S. aureus*: no regular disinfection of the stall, no regular replacement of stall bedding.

Animal Hygiene and Mastitis

Neave, et al (1969)

- Herds using a full hygiene milking routine (use of disinfectants, paper towels, or boiled cloths for washing each individual udder, the wearing of rubber gloves by the milker, and the pasteurization of teat cup clusters before each cow is milked, together with post-milking disinfectant teat dips) had a 45% reduction in new udder infection in one trial and a 58% reduction in the 2nd trial when compared with herds that practiced only washing with water and a shared cloth.
- Herds using a partial hygiene milking routine (same as full, but without the pasteurization of teat cup clusters) showed a 44% reduction in new udder infection when compared to control cows.

Pankey, et al (1987)

- Rate of IMI by major mastitis pathogens was reduced significantly by predipping plus good udder preparation compared with good udder preparation alone.

- Predipping reduced IMI due to environmental pathogens in each herd. Reduction in IMI with environmental pathogens ranged from 47% to 56%.
- This study suggests that the environmental pathogens cause new infections during milking. The inference is that the number of environmental pathogens on teats prior to milking is reduced significantly by predipping with an effective germicide, and consequently, the rate of new infections is reduced. It appears that environmental pathogens contaminate teat skin between mlkings but may or may not cause new infections between milkings.

Schreiner and Ruegg (2003)

- Udder hygiene scores (UHS) were significantly associated with leg hygiene scores (LHS).
- Linear somatic cell scores increased as UHS increased (dirtier udders).
- Significant differences in somatic cell scores were observed for clean (UHS scores of 1 [completely free of or has very little dirt] and 2 [slightly dirty]) versus dirty (UHS of 3 [mostly covered in dirt] and 4 [completely covered, caked-on dirt]) udders.
- There was a significant association between the prevalence of intra-mammary contagious pathogens in the milk and UHS but not LHS.
- The prevalence of intra-mammary environmental pathogens was significantly associated with UHS but not associated with LHS.
- Cows with UHS of 3 and 4 were 1.5 times more likely to have major pathogens (both contagious and environmental) isolated from milk samples compared with cows with hygiene scores of 1 and 2.
- The type of surface of the free-stall bed and the type of bedding used on that surface are likely to have a large influence on UHS but probably have less influence on LHS.
- Manure management systems, frequency of cleaning of barn alleys, and the ease of movement of cattle are likely factors that have a larger influence on LHS than on UHS.

Zarkower and Scheuchenzuber (1977)

- Pre-milking washing and drying of teats with iodine solution had no effect on total colonies, staphylococci, streptococci, gram-negative lactose fermenters and gram-negative lactose non-fermenters on the teat apex as compared to unwashed teats.
- When washed and dried thoroughly (with special care to include the teat orifice area), total number of colony-forming units was decreased significantly.

Zdanowicz, et al (2004)

- Udders of cows housed on sand had higher grid counts (dirtier udders) than those on sawdust.
- No clear correlation between udder cleanliness and teat end bacterial counts.

Somatic Cell Count (SCC) and Mastitis

Several studies have been conducted on the differences between herds that have low average SCC counts and herds that have high average SCC counts. Other studies look at the value of SCC count in determining intra-mammary infection status in herds. High SCC is correlated with decreased milk production. SCC is measured both with a bulk tank sample (BTSCC) and with individual milk samples from each cow. BTSCC can be a good indicator of a herd's general udder health status, with high BTSCC generally indicating a problem with contagious mastitis. Herds with lower BTSCC have lower subclinical mastitis and better general udder health. However, the presence of leucocytes in the udder helps protect it from getting other mastitis, therefore low SCC appears to predispose cows to getting environmental mastitis. By looking at individual cow SCC over a period of several months, patterns can be established for each cow. Spikes in individual cow SCC usually indicate environmental mastitis and are often short in duration. When SCC is done on a monthly or other low frequency basis, these spike may be missed.

Thus typical BTSCC cannot generally be used to diagnose environmental mastitis at the herd level unless it is pervasive and persistent.

SCC and Milk Yield

Barkema, et al (1998b)

- As bulk milk somatic cell count (BTSCC) decreased, milk production increased ($P<0.0001$). Herds with a low BTSCC had a mean cumulative fat corrected milk production during 305 d of lactation of 8589 kg compared with 8072 kg for herds with a high BTSCC.

Deluyker, et al (1993)

- Both elevated SCC and clinical mastitis were associated with milk yield losses.
- The milk yield loss associated with clinical mastitis represented 5% of yield in the first 119 d postpartum.
- A 6% yield loss was associated with a mean SCC of 383,370 cells/ml, compared with a mean SCC of 47,465 cells/ml.

Raubertas and Shook (1982)

- Regression coefficients for the average $\log_e$ of SCC were negative and highly significant for all lactations, indicating that increased average log cell count is associated with reduction in yield. Coefficients become larger with lactation number through the first three lactations.
- Yield loss per unit increase in average $\log_e$ cell count was 135 +/- 20 kg in first lactation and 270 +/- 30 kg for all other lactations.
- These relationships were linear indicating that loss per unit increase in actual cell count is greatest when cell count is low.

Hortet and Seegers (1998)

- At test-day level (milk production on the day of testing), the average trend was a loss of 0.4 kg of milk in primiparous cows and 0.6 kg in multiparous, by each 2-fold increase of SCC above 50,000 cells/ml.
- At the lactation level (cumulative milk production over the lactation), the average trend was a loss of 80 kg of milk in primiparous and 120 kg in multiparous, by each 2-fold increase of the geometric mean of SCC above 50,000 cells/ml.
- Protein content of milk showed a small increase of 0.15 g/kg (at the test-day level) while fat content showed a small decrease of 0.20 g/kg (both at the test-day and at the lactation level), by each 2-fold increase of SCC.

Salsberg, et al (1984)

- One unit increase in the $\log_e$ of the geometric mean of the somatic cell count was associated with a loss of 247 kg of 305 day milk production.
- One unit increase in the $\log_e$ of the 24 hour somatic cell count was associated with a decrease of 0.65 kg of test day milk production.

Dohoo, et al (1984)

- A unit increase in the log count of SCC resulted in a loss of 1.44 kg of milk at test day.

The Value of SCC in Determining Intramammary Infection Status

DeHaas (2004)

- Clinical mastitis can be predicted better by SCC patterns than by the average of 200,000 cells/ml in lactation.
- Short peaks in SCC are associated with clinical *E. coli.*

- Long increased SCC is associated with *Staph. aureus.*
- No pattern for streptococcus was shown.

Deluyker, et al (1993)

- In a low SCC herd free of *Staph. aureus, Strep. agalactiae* or *Strep. dysagalctiae*, cows with clinical mastitis were characterized by a high SCC prior to clinical mastitis diagnosis; SCC increased further around the time of diagnosis and returned to high premastitis counts after about 10 d following the end of treatment.

Hogan, et al (1988)

- Rates of total clinical mastitis were significantly correlated with bulk tank milk SCC (82.3% were environmental).

Smith, et al (1985b)

- SCC counts from individual or bulk tank counts are of questionable value for surveillance of environmental mastitis. This is because IMI are of short duration, and percent quarters infected at any time is generally not great.

Suriyasathaporn, et al (2000a)

- Very low somatic cell counts during the udder inflammation-free state (no mastitis) are associated with increased risk of clinical mastitis.

Peeler, et al (2003)

- The association between quarter somatic cell counts (QSCC) of milk and the risk of clinical mastitis (CM) was investigated in a one year study on three dairy herds in Somerset, UK.
- QSCC was categorized and the risk of CM occurring in the month after the QSCC was examined.
- When all cases of CM were considered, quarters with SCC 21,000 – 100,000 cells/ml had reduced odds and quarters with SCC > 200,000 cells/ml had over three times the odds of CM compared with QSCC 1,000 – 20,000 cells/ml.
- When only coliform CM were investigated, quarters with SCC 6,000 – 200,000 cells/ml had reduced odds of coliform CM compared with QSCC 1,000 – 5,000 cells/ml, and SCC > 200,000 cells/ml were not significantly different from the baseline.
- When *S. uberis* CM were investigated, quarters with SCC > 200,000 cells/ml had more than three times the odds of *S. uberis* CM compared with QSCC 1,000 – 20,000 cells/ml.
- QSCC < 21,000 and > 200,000 cells/ml are associated with increased odds of CM in the following 4 – 6 weeks: this association may be pathogen specific.

Zadoks et al, 2001.

- SCC was not associated with the risk of infection with S. uberis
- low SCC was associated with a lower risk of infection with S. aureus

Differences in Mastitis Between Low and High SCC Herds – Types of Bacteria

Barkema, et al (1998a)

- The mean incidence rate of clinical mastitis (IRCM) was approximately equal for herds in the low (SCC <=150,000/ml), medium (SCC 150,000 to 250,000) and high (SCC 250,000 to 400,000) bulk milk somatic cell count (BTSCC), but the pathogens were different and the severity of the disease was higher at the lowest BTSCC.
- The IRCM caused by *Strep. agalactiae, Strep. dysgalactiae* or *Staph. aureus* was lower for herds in the low BTSCC category than for herds in the medium or high BTSCC categories.
- Mixed cultures and contaminated samples were found less often in herds in the low BTSCC category than in herds in the high BTSCC category.

- The IRCM caused by *E. coli, Klebsiella* spp., *Pseudomonas* spp., and culture negative was higher for herds in the low BTSCC category than in the medium or high categories.
- The IRCM for cows that were reported by the farmer to be systemically ill was higher for herds in the low BTSCC category than for herds in the medium and high BTSCC categories.

Erskine, et al (1988)

- The incidence of clinical coliform (environmental) mastitis was significantly higher in the low SCC herds, but the incidence of clinical mastitis attributable to *Str. agalactiae* and *S. aureus* (contagious IMI) was significantly higher in the high SCC herds.

Hogan, et al (1989b)

- In a study of nine well managed herds with low somatic cell counts, a total of 646 clinical cases of mastitis were diagnosed. Coliforms, bacteriologically negative and environmental streptococci accounted for 82.3% of these cases, while contagious mastitis pathogens accounted for only 3.4% of the clinical cases.

Hutton, et al (1990)

- The only significant difference in the prevalence of intra-mammary infection major pathogens between high and low SCC herd groups was the pathogen *Staph aureus*. Eight times more cows had *S. aureus* in high than in low herds.

Jasper, et al (1975)

- Case histories of herds in California with coliform mastitis problems showed varying probable reasons for the problem.
- One herd's coliform mastitis problem coincided with their decrease in contagious mastitis problems.

Differences in Mastitis between Low and High SCC Herds – Management

Barkema, et al (1998b)

- Postmilking teat disinfection and dry cow therapy were practiced most frequently with herds with low bulk milk somatic cell count (BMSCC).
- For herds with a low BMSCC, more attention was paid to hygiene and detail than was paid to these areas for herds with medium or high BMSCC.
- Cubicles, drinking buckets and cows were cleaner in herds with a low BMSCC

Barkema, et al (1999)

- 300 Dutch dairy herds were studied for management style and its association with BMSCC.
- Cluster analysis was used to identify groups of farmers who had similar management styles for the prevention of mastitis – two management styles (clusters) were identified as clean and accurate, and quick and dirty.
- The relationship between clusters and BMSCC was high, but the relationship between clusters and mastitis was weak.
- Farms with herds that had a low bulk milk SCC had better hygienic conditions than those farms with herds that had a high bulk milk SCC.

Hutton, et al (1990)

- Low SCC herds (greatest % of animals with SCC <= 283,000 cells/ml) had lower moisture content of cow bedding than "high" SCC herds, however the prevalence of non-contagious mastitis was similar between low and high groups, thus it is not clear how drier bedding relates to lower SCC.

Schukken, et al (1990)

- Risk factors associated with the mastitis rate in herds with low bulk tank SCC included the use of mats in cubicles, and the percentage of dirty cubicles. Rubber mats were generally associated with a moist surface giving an environment that may support bacterial growth. Percentage of dirty cubicles was correlated to the rate of mastitis and also correlated to the cleanliness score of the cows.
- A high frequency of cubicle disinfection per month (with formalin) was associated with higher mastitis, possibly by causing skin irritation and lesions which are predisposing to clinical mastitis.

Schukken, et al (1991)

- Presence of rubber mats in herds with low bulk tank SCC was associated with an increase in the incidence rate of both *E. coli* and *S. aureus* mastitis.
- More frequent cleaning of manure by hand from the cubicle was associated with lower incidence rate of *E. coli* mastitis.
- Greater amount of bedding in cubicles of the lactating herd was associated with lower incidence rate of both *E. coli* and *S. aureus* mastitis.

Other Mastitis Issues

Other issues that may affect intramammary infection in dairy herds include stage of lactation and the dry period, parity (number of lactations), milking and milking machine factors including the use of post milking dips, teat end roughness and callosity, seasons of the year, nutrition, and housing conditions other than bedding.

Stage of Lactation

Barkema, et al (1998a)

- The highest incidence rate of clinical mastitis (IRCM) was in early lactation. Peak incidence around calving was higher in heifers than in older cows: >30% of the cases of clinical mastitis in heifers occurred during the first 14 d of lactation, but, in cows, this prevalence was at 13%. After the 2nd wk of lactation, the IRCM was higher in cows than in heifers.

Bartlett, et al (1992)

- A greater prevalence of environmental streptococcal infection was associated with herds that had increased number of days dry.

Hogan, et al (1989b)

- Rates of clinical mastitis were highest the first 90 d and decreased throughout lactation.
- Rates of clinical cases was highest the week following calving for each of coliform, environmental streptococcal and bacteriologically negative clinical cases.

Erskine, et al (1988)

- Low SCC herds had a high incidence of clinical mastitis during the first month of lactation, while clinical mastitis in high SCC herds tended to be uniform during the entire lactation period.

Peeler, et al, (2000)

- The rate of clinical mastitis decreased with a dry period of <40 days.

Smith, et al (1985a)

- Dry treatment significantly influenced the rate of environmental streptococcal IMI during the dry period. Rate of strep IMI was highest in cow groups not dry treated (6 to 7 times higher).

- However, for coliform mastitis, after adjusting for parity and season, there was little or no indication that any of the treatments (dry cow therapy, immunization, artificial infusion and combinations thereof) including immunization significantly altered the rate of coliform IMI during the dry period.

Smith, et al (1985b)

- Rate of coliform IMI was highest in first 76 days of lactation and decreased progressively as lactation advanced.
- Rate of streptococcal IMI was twice as high as coliform IMI and decreased as lactation advanced, but not as markedly as coliform IMI.
- Rate of coliform IMI in the dry period was 3 to 4 times higher than the rest of lactation.
- Rate of streptococcal IMI in the dry period was 1.6 times higher than rest of lactation.
- Dry cow therapy had an effect on streptococcal IMI, but not coliform.

Todhunter, et al (1995)

- Rate of new environmental streptococcal IMI was highest during the 1st month of lactation, and were highest in that period for cows in lactation >= 4 and heifers.
- The rate of IMI declined from 31 to 150 DIM for all cows.
- The rate of IMI further declined from 151 DIM to drying off for cows in 1st or 2nd lactation, but rates of new infection in late lactation increased for cows in 3rd and 4th lactation compared with rates at 31 to 150 DIM.

Parity

Barkema, et al (1998a)

- The incidence rate of clinical mastitis increased as parity increased.

Smith, et al (1985a)

- Parity group had an influence on IMI. Heifers had less coliform IMI than 2nd and 3rd lactation.

Smith, et al (1985b)

- Rate of coliform IMI was approx 3x as high in multiparous cows as heifers in first lactation.
- Parity had an effect on both coliform and streptococcal IMI. Rate of both increased approximately 5 times from 1st lactation to lactation 6 or greater.

Zadoks, et al (2001)

- Rate of IMI by *S. uberis* and *S. aureus* are lower in first and 2nd parity than in older cows.

Milking and Milking Machine Factors

Barkema, et al (1999a)

- Milking machine factors were associated with the incidence rate of clinical mastitis (IRCM) caused by *E. coli*, *Streptococcus dysgalactiae*, and *Streptococcus uberis.* As milking vacuum pressure increased, prevalence of IMI increased.
- Postmilking teat disinfection was associated with an increased overall IRCM and IRCM caused by *E. coli*, especially in herds in the low BTSCC category.

Bartlett, et al (1992)

- A greater prevalence of coliform infection was associated with herds that had a comparatively large amount of milk left in the udders after being milked, herds with longer milking times, herds that used running water to clean cows before milking and herds with more liner slippage.

- A lower prevalence of environmental streptococcal infection was associated with herds that used individual rags or cloths for drying udders.

Eberhart and Buckalew, (1977)

- The level of infections with streptococcal species other than *Str. agalactiae*, which was initially low (1.8%), has increased to 6.3% over the years since post-milking teat dipping and dry-cow therapy were introduced in the Pennsylvania State University dairy herd.
- Comparison of incidence of clinical mastitis over several years indicates that the incidence was not appreciably reduced by the use of teat dipping and dry cow therapy, but that there were changes in the types or organisms isolated. Streptococcal species other than *Str agalactiae* and gram-negative organisms became the cause of about two-third of the clinical mastitis.

Hogan, et al (1988)

- Bulk tank milk bacterial counts were associated with the number of quarter-milkings that liners were used. Liners used greater than 1200 quarter-milkings were associated with higher total bacterial and staphylococcal counts than were liners used less than 1200 quarter-milkings. This could be caused by teat skin bacteria adhering to the worn surface of the liners.

Jasper, et al (1975)

- Case histories of herds in California with coliform mastitis problems showed varying probable reasons for the problem.
- Two years after virtually eliminating contagious mastitis problems, one herd began to have trouble with acute coliform mastitis. In this case, a batch of liners was defective and rapidly became cracked. The problem disappeared almost immediately after the liners were replaced.
- The problem in another herd illustrates that bacterial build-up and infection can also occur through the efforts of man that change the ecologic environment. In this instance, chlorhexidine of unknown and imprecise concentrations was being used to disinfect teat cup clusters between cows and between milkings. The chlorhexidine had effectively eliminated the natural microbial competition and had left the field free for abundant growth of pseudomonas. Exposure to the heavily colonized liner during milk was sufficient to bring about quarter infections.

Neave, et al (1969)

- Large differences in new infection rates between herds using full hygiene systems to control mastitis were most probably due to milking machine differences that result in an increase in infection during milking, i.e. vacuum reserve, air bleed, pulsation characteristic, milk lift and inflation design.

Peeler, et al, (2000)

- Survey of management practices of British dairy herds with low somatic cell count (average 76,000 cells/ml)
- The following milking variables were associated with increased rate of clinical mastitis:
  - Herds that always practiced post milking teat disinfection
  - Herds that changed the teat liner at > 6000 or more milkings
  - Herds where there were cows leaking milk on entering the parlor
- The following milking variables were associated with decreased rate of clinical mastitis
  - Herds that used a rotary parlor
  - Herds that used a confinement yard (loafing) after milking
  - Herds using automatic cluster removal.

Zarkower and Scheuchenzuber (1977)

- Use of a post-milking iodophor teat dip significantly reduced the total bacterial and staphylococcal populations but no effects were noticed on the streptococcal bacteria counts.

Teat Ends

Neave, et al (1969)

- In herds practicing full hygiene a significant relationship was found between the new infection rate and the number of cows with teat lesions.

Neijenhuis, et al (2001)

- In the within-cow analysis (teat end callosity thickness - TECT and roughness - TECR compared between quarters with mastitis and lateral quarters of the same cow without mastitis), TECT was significantly higher in the mastitic quarters than in those without clinical mastitis. There was no difference in TECR.
- In the between cow analysis (cows with mastitis were paired with similar cows without mastitis based on parity and date of calving), clinical mastitis cows had thicker, and more frequently rough, callous rings on their teat ends than cows that did not have clinical mastitis, both before and after the clinical mastitis occurred, if it occurred between the 1st and 6th month of lactation. On the other hand, cows with clinical mastitis in the first month of lactation showed less TECT and TECR during lactation than other cows.
- Clinical mastitis cases which were culture-negative or caused by less frequently found pathogens like yeast, *K. pneumoniae* and *E. aerogenes* were associated with higher teat end callosity, while clinical *E. coli* mastitis was associated with less TECT.

Zadoks, et al (2001)

- Teat end roughness and extreme teat end callosity increased the rate of *S. aureus* mastitis but not *S. uberis* mastitis.

Seasonality

Hogan, et al (1988)

- Rates of clinical mastitis differed among seasons of the year and were associated with bulk tank milk somatic cell counts.
- Rates of total and coliform clinical cases were higher during summer than spring.

Hogan, et al (1989b)

- Mean rate of clinical mastitis cases was highest during summer and decreased throughout fall and winter to a low in spring.
- Rates of coliform and bacteriologically negative clinical cases were highest during summer, lowest during spring.
- Rates of clinical mastitis caused by environmental streptococci did not differ among seasons of the year.

Erskine, et al (1988)

- The peak incidence of clinical coliform mastitis was recorded during August. Peak percentages of clinical mastitis caused by other environmental mastitis organisms were recorded in July or August, and the peak incidence of contagious pathogens was in June, July and August.

Smith, et al (1985a)

- Season of the year has an influence on IMI. Coliform IMI was lower in winter (Dec, Jan, Feb) and fall (Sep, Oct, Nov) than in spring (Mar, Apr, May) and summer (Jun, Jul, Aug).
- Parity group had an influence on IMI. Heifers had less coliform IMI than 2nd and 3rd lactation.

- After adjusting for parity and season, there was little or no indication that any of the treatments (dry cow therapy, immunization, artificial infusion and combinations thereof), including immunization significantly altered rate of coliform IMI during the dry period.

Smith, et al (1985b)

- Rate of coliform IMI was elevated by a factor of 3 during summer and the effect was primarily associated with multiparous cows.

Todhunter, et al (1995)

- Rates of environmental streptococcal IMI during the dry period and during lactation were greatest during summer.

Nutrition

Barkema, et al (1999a)

- Nutrition was associated with the incidence rate of clinical mastitis (IRCM) caused by *E. coli, Streptococcus dysgalactiae,* and *Streptococcus uberis.* The presence of minerals in the diets of lactating cows was associated with a decreased IRCM caused by *S. dysgalactiae* and *S. uberis.* When lactating cows were fed corn silage, a lower overall IRCM and IRCM caused by *S. uberis,* and a higher IRCM caused by *E. coli* were observed.

Peeler, et al, (2000)

- Offering fresh feed after both milkings decreased the rate of clinical mastitis.

Suriyasathaporn, et al (2000b)

- A review of the role of ketosis resulting from negative energy balance in the risk of mastitis.
- Udder defense mechanisms are reduced in cows with ketosis, resulting in increased risk of mastitis.

Weiss, et al (1997)

- Cows were assigned to one of three treatments at 60 d before anticipated calving:
  - Treatment 1 – 100 IU/d of supplemental vitamin E during the dry period and 100 IU/d during the first 30 d of lactation.
  - Treatment 2 – 1000 IU/d of vitamin E during the dry period and 500 IU/d during lactation.
  - Treatment 3 – 1000 IU/d of vitamin E during the first 46 d of the dry period, 4000 IU/d during the last 14 d of the dry period, and 2000 IU/d during lactation.
- The percentage of quarters with new infections at calving was not different (32.0%) between cows receiving treatments that contained low and intermediate concentrations of vitamin E but was reduced (11.8%) in cows receiving the high vitamin # treatment.
- Clinical mastitis affected 25.0, 16.7, and 2.6% of the quarters during the first 7 d of lactation for cows receiving the low, intermediate, and high vitamin E treatments, respectively.

Housing Other than Bedding

Barkema, et al (1999a)

- A lower incidence rate of clinical mastitis caused by *E. coli* was associated with complete slatted floors and alleys, and lower animal density.

Barrett, et al (2005)

- Herds with less than 110 cubicles per 100 cows were more likely to experience environmental mastitis.

Bartlett, et al (1992)

- A greater prevalence of coliform infection was associated with herds that used freestalls in the winter.
- A greater prevalence of environmental streptococcal infection was associated with herds that housed animals in tie stalls.

Peeler, et al, (2000)

- Survey of management practices of British dairy herds with low somatic cell count (average 76,000 cells/ml) showed the following housing variables lead to increased rate of clinical mastitis: lactating cows housed in straw yards compared with cubicles, dry cows housed in straw yards compared with cubicles and access of milking cows to outdoor yards (when housed).

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