The Coliform Mastitis Enigma

D. E. Jasper, *D.V.M., Ph.D.,*
Department of Clinical Pathology
University of California, Davis, CA 95616.

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Observations on coliform mastitis extend for more than 50 years but it has been only recently that dairymen and scientists have become really concerned about the problem. Several trends in the dairy industry are probably responsible.

Success in controlling streptococcal and staphylococcal mastitis leaves more quarters at risk to coliform mastitis. A herd of 100 cows with 200 quarters infected with other pathogens would have 200 quarters at risk to coliform mastitis. A herd without other infections would have 400 quarters at risk to coliform mastitis. Therefore, in the latter herd, twice as many coliform infections could be expected even though the rate of infection per quarter at risk remained unchanged. The dairyman with many cases of streptococcal and staphylococcal mastitis may not know that some cases are due to coliform bacteria. They are lost and unrecognized among many cases of mastitis due to the cocci. But the dairyman with a generally uninfected herd is more likely to know that coliform bacteria are causing mastitis.

Many herds are now kept in form of confinement housing which promotes high populations of coliform bacteria in the bedding so that risk of infection becomes greater. Milking systems milk more cows and may themselves act as a source in spreading infection.

The demonstration by Schalm and others that increased cell counts in quarter milk protects that quarter from infection due to coliform or other bacteria has lead to speculation that complete freedom from other infections would create a dangerous condition of susceptibility to coliform infection in a herd. Therefore, it is reasoned, a certain unspecified level of infection should remain in the herd, or that bulk tank cell counts should not be reduced below 500,000 cells, or some other magic level. I consider this to be erroneous thinking, for the protection provided by other infections, or by increased cell counts, is always on a quarter basis, never on a cow or on herd basis. If 10 or 80 percent of quarters have high cell counts, 10 or 80 percent quarters will have some protection against coliform bacteria. To follow this reasoning to its logical end we should strive for 100 percent quarter infection with other bacteria in order to protect against coliform infections. I take the opposite viewpoint. We should strive for zero infection with all bacteria, including coliform mastitis. It is the latter objective with respect to coliform mastitis that we are concerned with today.

Our program begins with consideration of the environment and increased evidence from Dr. Francis that environmental changes modify bedding populations in ways that affect new coliform infection rates. Dr. Francis found that changes in cow behavior with the season also influence bedding counts. This knowledge affords an opportunity to modify the environment in a favorable fashion, or to counteract an unfavorable environment by deflecting back-jets in the milking equipment, as Dr. Bramley suggests.

Our understanding of the role of somatic cells in protection against mastitis has been greatly enhanced by Dr. Schalm. However, detailed cellular and tissue changes in pathogenesis are best revealed in the very interesting electron microscopy studies of Dr. Hill. In addition to all this Dr. Grootenhuis will show us that the somatic cell count in milk of first lactation daughter groups provides a practical parameter for selection for resistance to mastitis. I believe genetic selection for resistance to mastitis has been overlooked and I hope the paper of Dr. Grootenhuis will stimulate much more work along this line.

Of considerable interest is the paper by Farnsworth which describes effectiveness of teat end sealant in protecting against coliform mastitis under conditions of high risk.

Selection of therapy for coliform mastitis has always been a problem and it is encouraging to note that Drs. Verheijden, Flipovic, and Muller are suggesting promising treatment approaches by which to improve our recovery rates.

Milking equipment has long been implicated in mastitis, and its role in causing or avoiding mastitis and teat lesions will be discussed by Drs. Thompson, Sagie, Hamann and Sieber. The very interesting report of Heckman and Noorlander show by electron microscopy that minute imperfections in the surface of inflations may harbor coliform and other bacteria. When to this list of contributors we add those of Drs. van den Heever and Saran we can really look with optimism toward new understanding and new knowledge of technology to reduce mastitis problems of all kinds.

Several reports have suggested that bedding coliform populations are an important factor predisposing to coliform mastitis.[1-5]

With this background I wish to discuss observations, materials and methods made by Dr. E. J. Carroll and myself on several dairies typical of our area of California and which we have reported to the National Mastitis Council.[6]

Paper presented at the International Congress on Cattle Diseases, Tel Aviv, Israel, October, 1980.

Approximately one Kg samples of bedding were obtained from several areas in corrals and free stalls where cows had been lying, and placed in plastic bags. These were refrigerated until the next day, at which time they were processed.

Bacteria counts: The contents of each plastic bag were mixed thoroughly. A 10 gm sample was placed in a Waring blender along with 90 ml of sterile 0.85% NaCl solution and blended for one minute. Ten ml were then transferred to another 90 ml of saline by pipette. Serial 1:10 dilutions were then made out of 8-10 tubes depending upon the counts expected from that particular sample. A 0.1 ml aliquot of each tube or bottle was then transferred to 5.0 ml of MacConkey broth and these were incubated at 37°. Growth in the tube, as evidenced by acid production, was recorded and a loop of contents of each tube showing growth was streaked on the surface of Tergitol-7 agar (Difco). Colonies representing each morphological type on the surface of the Terigitol-7 plates were then streaked on to Triple-Sugar-Iron agar slants and incubated. Growth from these slants were placed on MRVP medium, motility and citrate agar and, when necessary, other confirmatory media such as ornithine decarboxylase and urea media.

Total coliform counts are given as calculated from the last dilution showing growth in MacConkey broth and of individual coliform species calculated from the last dilution from which that particular organism had been isolated.

After gaining experience, it was found that reliable identification of *E. coli,* Klebsiella and Enterobacter species could be made from colony morphology on Tergitol-7. Additional verification was also made with the API system (API 20E, Analytab Products Plainview, N. Y.).

Results

Effect of Physical Condition

At the outset, it was noted that what might appear to be excellent bedding visually, may be the most unsatisfactory from the standpoint of bacterial numbers and kinds per unit weight. Conversely, what would appear to be the poorest bedding visually might have the lowest bacteria counts (Tables 1,2). Old sawdust bedding well-mixed with manure has been observed to have a count of about 10^5 while the new 3-day old replacement sawdust bedding already had a count of 10^7 coliform bacteria.

Table 1. Coliform Bacteria Counts of Samples Described as Wet, Sloppy or Muddy Manure.

Number	%	Counts/gram	Remarks
2	10	10^3	Only three samples had
15	75	10^4	organisms other than
3	15	10^5	*E. coli* present.
20			

Table 2. Coliform Bacteria Counts of Samples Described as "Dried Manure".

Number	%	Counts/gram	Remarks
3	4.3	10^2	All *E. coli*
10	14.4	10^3	Only 1 sample with Klebsiella and Enterobacter
21	30.4	10^4	All *E. coli*
24	34.7	10^5	2 samples with Klebsiella or Enterobacter
10	14.4	10^6	1 sample with Klebsiella and Enterobacter
1	1.4	10^7	All *E. coli*

It is seen that both very wet and very dry samples, even when composed largely of manure, have total coliform counts clustering around 10^4-10^5 per gram. Such counts are not considered to be high and are unlikely to frequently cause mastitis problems. A few "dry samples" did, however, have high counts and were perhaps hazardous. Possibly they were not as "dry" as they appeared.

The counts are based on grams wet weight. However, it is recognized that wet material, although it may weigh more per unit volume, does not have the surface area that light, dry material has. It would appear that both the very wet and the very dry conditions do not favor the *growth* of coliform bacteria. It has been found that coliform counts of freshly voided bovine feces rarely exceed 10^5/gram wet weight using our cultural methods for counting. This is not a highly dangerous number of coliform bacteria as work has shown that mastitis tends to increase when coliform counts reach 10^6 or higher. [1,2]

From the standpoint of bacterial numbers, fully dried manure makes an entirely satisfactory bedding material.

Sawdust Bedding

Fresh sawdust was cultured after arrival at several dairies (Table 3). Numbers of bacteria in fresh sawdust were generally very low. *Klebsiella* organisms were not easily recovered when indigenous to fresh sawdust in low numbers and required enrichment of sawdust in a bacterial medium favoring growth of fastidious organisms. Although *Klebsiella* were not recovered from fresh sawdust at Dairy C, they were recovered from sawdust at other dairies and may well have been present in sawdust brought to Dairy C as well.

The sawdust bedding used on Dairy C was subject to a 12-month study. In October, 1977, all pens had been scraped to the dirt base and the herdsman reported there was an increase in their coliform mastitis rate. In November, 1977, all pens again were scraped to the dirt base onto which was to

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Table 3. Coliform Bacteria Counts of Fresh Sawdust.

Date	Total Count/gram Wet Weight	Remarks
Dairy C		
8-18-76	Less than 10^2	Citrobacter only
2-16-76	10^2	*E. coli* and Klebsiella
12-12-77	10^1	*E. coli* only
12-19-77	Less than 10^1	*Enterobacter agglomerans* on enrichment only
Dairy D		
11- 4-77	10^3	*E. coli,* Enterobacter, Klebsiella
Dairy ST		
2-15-77	10^3	Klebsiella, Enterobacter
Dairy BU		
3-18-78	10^2	Enterobacter only byplating; Enterobacter and Klebsiella on enrichment

be added fresh sawdust. The coliform counts of the dirt base (the newly scraped surface) were as in Table 4. Although counts were low at the time of sampling, they may have been much higher immediately after the pens were scraped. Samples were taken at intervals after sawdust was placed in the corrals on Dairy C providing an opportunity for observing changes with time in bacteria count of the sawdust bedding (see examples in Table 5).

The fresh sawdust added to the scraped corrals was found to have a count of only 10 bacteria/gram (10^1), all *E. coli.* However, it would appear that bacteria grow very quickly in

Table 4. Coliform Bacteria Counts of Corral Dirt Base on Which Fresh Sawdust will be Placed as Bedding. Consists of Dirt, Decomposed Sawdust and Dry Manure and is Very Compact and Dense.

Corral	Total Coliform Count/gram	Organisms and Count/gram
1	10^5	*E. coli* 10^5; Enterobacter 10^5 Klebsiella 10^4
2	10^3	*E. coli*
3	10^3	*E. coli*
4	10^4	*E. coli*
5	10^3	*E. coli*

Table 5. Coliform Bacteria Counts of Corrals after One Week with Sawdust present for Various Times. Weather = Rainy.

Corral	Remarks	Total	Organisms Present
1	Sawdust present one week. Very muddy and dirty	10^4	*E. coli,* Klebsiella
1	Sawdust present one week. Less muddy area under cover but sawdust is damp.	10^7	*E. coli,* Klebsiella 10^7
2	Sawdust present one week. Muddy sawdust and manure.	10^6	*E. coli,* Klebsiella 10^6
3	Sawdust present 2 days. Muddy sawdust and manure.	10^4	*E. coli,* Enterobacter 10^2
4	Sawdust present one day.	10^4	*E. coli,* Enterobacter 10^2

fresh sawdust if cows and feces are present and the corrals are not excessively sloppy. Counts of 10^4/gram were observed in 1 to 2 days. Counts of 10^7/gram were found within a week after the sawdust was placed in the corrals.

Additional evidence of the rate and level of growth of bacteria in sawdust was obtained from the survey data on Dairy C (Table 6). Counts of different coliform bacteria varied considerably from time to time. This is selected data only in that all the bedding samples were new in appearance and were described as fresh sawdust bedding material. It can be seen that very high total counts were usually found, including high counts of *Klebsiella* organisms. On 4-5-77, new sawdust was placed under cover in corral 1. Samples were obtained at intervals during the month. Within a week, counts of 10^7/gram were obtained.

Thus, in contrast to the wet, muddy manure which had low total counts (mostly *E. coli*), nearly new sawdust, which has a clean, pleasant appearance, can harbor very high numbers of potential udder pathogens and these numbers can be reached within a few days after sawdust is added to corrals or covered loafing areas (Figure 1).

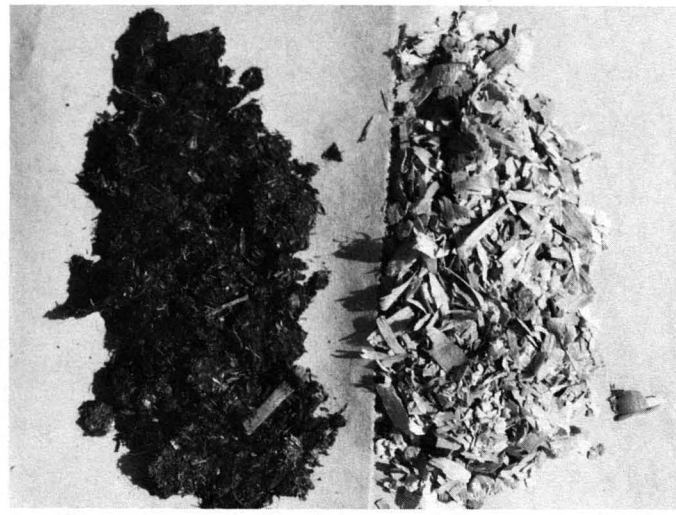

Figure 1. The "clean" shavings and sawdust had been used only 3 days but had a coliform count of approximately 10^7. The "old" bedding had a coliform count of about 10^5.

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Table 6. Coliform Bacteria Counts from Sawdust Bedding.*

Date	Corral	Remarks	Total Coliform Count/gram	*E. coli*	Kleb-siella	Entero-bacter	Citro-bacter
8-18-76	4	New 8-17	10^3	10^3	10^2	10^3	
11-11-76	1	Inside	10^8	10^8		10^6	
12- 7-76	1	Inside	10^8	10^7	10^8	10^5	
2- 7-77	5	Inside	10^8	10^8	10^7	10^7	
2-16-77	4	Outside	10^7	10^7	10^4	10^5	
3- 3-77	4	Outside	10^7	10^5	10^6	10^6	10^7
3- 3-77	3	Inside	10^7	10^6	10^7	10^7	
4- 6-77	1	Inside New 4/5	10^5	10^5			
4-11-77	1	Inside New 4/5	10^6	10^6	10^5	10^6	
4-13-77	1	Inside New 4/5	10^7	10^7	10^3	10^6	
4-18-77	1	Inside New 4/5	10^6	10^5	10^5	10^6	
4-28-77	1	Inside New 4/5	10^6	10^6	10^2	10^3	
5- 3-77	4	Outside New 4/29	10^8	0	10^7	10^8	
6- 9-77	4	Outside	10^6	10^6	10^5	10^5	

* Described as newly placed in the corral or barn within 3 days of time of sampling or as dated.

Laboratory Experiments with Sawdust.

Fresh sawdust used on Dairy C was mixed together with various amounts of fresh feces, saline, muddy manure or freshly voided urine, incubated at room temperature in the laboratory and coliform counts made at various times. Although differences in replicate counts indicated a rather large sampling error, several preliminary conclusions could be drawn.

The experiments demonstrated that levels of manure sawdust of approximately 1:4 resulted in an initial coliform count of 10^4/gram and rose to a maximum of 10^7/gram within 24 hours. Klebsiella organisms were rarely found in bovine feces and were difficult to demonstrate in fresh sawdust. However, counts of Klebsiella organisms indicated numbers of 10^5/gram were often present after 24 hours. Whether these organisms were originally in very low and undetectable numbers in the fecal inoculum or the sawdust at the outset could not be determined. Moisture alone (salt solution) added to sawdust did not significantly contribute to growth of coliforms in the sawdust presumably due to lack of available nutrients for the organisms. Bovine urine alone which was sterile at the start, also did not contribute to significant multiplication of coliforms in the sawdust. The strong ammonia smell of mixture suggested that alkalinity may have restricted growth of organisms. The combination of sawdust and feces on the other hand resulted in a 2-3 log increase in growth of all coliforms in the mixture and, significantly, this growth occurred within the first 24-48 hours.

Recycled Manure Bedding

In a previous report,[6] data were given which showed that the composting of manure solids reduced the coliform bacteria content and that the composted solids would make satisfactory bedding material. It was shown that composting effectively reduced coliform counts to low levels or to zero. However, if given the proper conditions of moisture and temperature, coliform bacteria would multiply to high numbers again whether from surviving organisms in the compost or from external contamination. Only the interior of the compost pile generally undergoes sufficient fermentative activity to raise temperatures to lethal levels for coliforms.

In a separate study, Allen, *et al.*[7] concluded that recycled manure solids composted for 3 weeks or more and allowed to air dry in the summer had several advantages over fresh manure solids. Coliform counts and moisture content were very low in such material and they felt that cows preferred the composted dry product to fresh manure solids. Keys[8] also observed that cows preferred 90% dry matter dehydrated manure solids to 29% dry matter manure solids or even 81% dry matter sawdust. It is interesting to note that the cows chose the bedding least likely to give rise to coliform mastitis.

Although coliform mastitis occurs most commonly in cows kept in confinement, it can also appear in cows which are pastured except for milking time. Three successive coliform mastitis problems were investigated in pastured cows in New Zealand. One outbreak was due to high

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populations of coliform bacteria on inflations, one was due to treatment of all cows for streptococcal and staphylococcal mastitis without adequate disinfection of teat ends and one problem ceased after milking machine function, husbandry faults and defective milking sanitation were corrected.[9]

Teat skin and milking machine inflation populations of coliform bacteria can be greatly reduced by flushing with iodophor disinfectants or heat treatments prior to or between milkings.[10],[11] Automation of teat cup flushing between milkings might help to reduce coliform mastitis by eliminating carry-over of coliform bacteria from one cow to the next.

Obviously we don't yet have all the answers to the coliform mastitis problem but our conference here today should leave each one of us just a little better prepared to cope with the problem in our own areas of responsibility.

Conclusions

Dried manure or composted dried manure solids make excellent bedding as long as they are kept dry and not damp with urine and feces. Some dirt mixed with dry manure solids may improve physical characteristics and help to maintain low bacterial counts. Sawdust mixed with shavings seems to promote growth of coliforms, especially *Klebsiella* bacteria when moist and contaminated with manure. Management of bedding materials may therefore be an important factor affecting incidence of coliforms mastitis.

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CORRECTION

Dear Sir,

In the paper we submitted for publication in last year's Bovine Practitioner, there are, unfortunately, a few mistakes in the legends of the figures. We would greatly appreciate the opportunity to enter a short notice in this year's Bovine Practitioner, for the figures now suggest the opposite of what was intended as to the effect of the commercial preparations.

The notice should read:

"In our paper: Investigations on Spontaneous and Glucocorticoid Induced Glucosuria in the Bovine Animal (Bovine Practitioner No. 14, 1979, 94-98), the legend of figure 1 is incomplete, and the legends of figures 2 through 5 are mixed up. The correct lengends read as follows:

Figure 1: Average values of blood and urine glucose concentrations (mg/dl; mmol/1) and of eosinophil counts (% of initial values) following glucocorticoid treatment in four test groups.

--------- Preparation A
_____ Preparation B
-.-.-.-.-. Preparation C
..... Preparation D

Figure 2: Absolute glucose losses (g) of five cows following administration of preparation D

Figure 3: Absolute glucose losses (g) of five cows following administration of preparation A

Figure 4: Absolute glucose losses (g) of five cows following administration of preparation B

Figure 5: Absolute glucose losses (g) of five cows following administration of preparation C

Munich, June 1980

D. SCHILLINGER W. KLEE

We regret to have caused such an inconvenience.

Yours sincerely,

W. Klee
II. Medizinische Tierklinik
Universitat Munchen
W. Germany

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