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**Transport and Propagation of Organisms
to and through the Teat Canal during
and between Milkings**

N.R. Scott, D. Galton, E. Maltz, D.J. Aneshansley

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Transport and Propagation of Organisms To and Through the Teat Canal During and Between Milkings

Investigators' Names
(Principal listed first)

Investigators' Institutions

N. R. Scott

Cornell University

D. M. Galton

Cornell University

Ephriam Maltz

Volcani ARO

D. J. Aneshansley

Cornell University

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Signature
Principal Investigator

Signature
Institution's Authorizing Official

Donna Jean Garrett
Senior Grant and Contract Officer

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למדעי החקלאות
בית-דגן

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Abstract

Mastitis remains a serious problem for the dairy industry and greatly influences the profitability of dairy farming. Propagation of bacteria through the teat canal requires that bacteria be present at the teat end and that conditions exist which are conducive for penetration of organisms. The milking machine may be involved as a vector in transfer of mastitis-causing organisms or as a factor in creating conditions that make the teat more susceptible to bacterial penetration.

The major objectives of this research project have been to quantitatively define mechanisms for transport of mastitis-causing pathogens through the teat canal and to quantify the effects of the liner on forces exerted on the teat and their potential effect on reverse pressure gradients (across the short milk tube and across the teat canal) leading to the transport of organisms through the teat canal.

Major achievements of this project are:

- 1) Reverse pressure gradients have been shown to exist under certain milking conditions. Liner slippage and large influx of air flow in the short milk tube at no milk flow at the end of milking have been implicated as possible causes for the development of intramammary infections due to the movement of pathogens through the teat canal. In addition, our findings suggest that the method of machine removal affects the potential occurrence of RPGs. The direct recovery of organisms from the teat sinus following liner slippage, large acute air admissions, and machine removal further substantiates that RPGs occur and are likely to be a primary factor in organism transfer through the teat canal
- 2) Both experimental and analytical results suggest that diffusion may be a mechanism of pathogen transport into the teat canal. However, it is unlikely that the diffusion mechanism alone would support pathogen transport into the teat's cistern during the normal interval between milkings. A more likely mechanism is bacterial growth through the teat canal, combined with the diffusion process.
- 3) Measurements of effective teat loading during machine milking provides a useful means to characterize the effect of basic liner/teat interactions. Our studies have quantitatively characterized the "squeeze" phase of the pulsation cycle and provide evidence that the load applied by the liner actively aids in the redistribution of blood and serum within the teat to relieve teat tissue from the applied vacuum which induces blood and serum pooling within teat tissue which can affect milk removal.
- 4) Results from field studies in six Israeli kibbutzim indicate that the milking system, especially the teatcup assembly can affect teat conformation and possibly the incidence of mastitis. A "wide" liner (23-25 mm) compared with the "narrow" liner (19-21 mm) causes greater increases in teat dimensions and an apparent negative effect on teat health.

These achievements correlate well with the objectives of the original research proposal. Specifically, the experimental verification of reverse pressure gradients across the teat canal is a major contribution to the understanding of the importance of the milking machine under conditions of liner slippage, large acute air admissions, and machine removal to the transport of pathogens through the teat canal, leading to the incidence of mastitis. Also, the understanding of pathogen transport through the teat canal, via a combination of diffusion and bacterial growth, is a major contribution to understanding mechanism of pathogen transport.

These findings provide a better understanding of mechanisms involved in bacterial transfer in penetration and provide the basis for design of machine systems and practices which will reduce the potential for organism transport through the teat canal. The implementation of systems to reduce organism transport on the basis of these findings can have a very significant impact on reducing the incidence of mastitis and, thereby, the substantial economic losses as a result of this disease.

**TRANSPORT AND PROPAGATION OF ORGANISMS
TO AND THROUGH THE TEAT CANAL DURING
AND BETWEEN MILKINGS**

BARD PROJECT NO. US-705-83

OBJECTIVES

The major objectives of the research are to quantitatively and qualitatively define mechanisms for transport of mastitis-causing pathogens through the teat canal and to quantify effects of liner forces exerted on the teat and their potential effect on reverse pressure gradients across the short milk tube and across the teat canal. Four specific sub-objectives are:

1. Measure the forces exerted on the teat by the liner to determine how liner forces vary between liners and cows during milking.
2. Determine whether correlations exist between different liner forces and general milking characteristics such as milking time, flow rate, etc.
3. Determine and quantify machine milking parameters that cause reverse pressure gradients across the teat canal.
4. Analyze both theoretically and experimentally mechanisms for transport of mastitis-causing pathogens through the teat canal.

DESCRIPTION OF RESEARCH RESULTS

Forces Applied by the Liner on the Teat (Objectives 1 and 2)

In modern milking systems, a liner (or "inflation") is the component in direct contact with the teat during milk removal. The benefit of using liners is generally attributed to the "squeeze" phase of the pulsation cycle - - when the liner collapses and puts a compressive load on the teat. This load actively aids in the movement of blood and serum out of the teat and relieves teat tissue from the applied vacuum which induces blood and serum pooling within the tissue and consequently may affect milk removal. There is no general agreement as to what constitutes an "adequate" liner as compared with a "poor" liner.

The development of a method to measure the load exerted on the teat by the liner provides the potential for quantifying and understanding liner action. Such measurements can be used to assess current liner designs and determine the effects of changing various properties. Knowledge of the teat surface load is also necessary as boundary conditions in analytical models to predict teat tissue stresses (Gates, et al, 1986).

The availability of modern miniature electronic pressure transducers that are linear, accurate, and durable enabled us to construct a transducer to measure the load (force/unit area) on the teat surface caused by the liner during milking. Simultaneous use of two transducers gives the net load on the teat surface within and perpendicular to the plane of liner collapse. Similar measurements at various locations along the length of the teat can be used to develop a "map" of effective teat load over the teat surface. The transducer developed to measure effective teat load is given in detail by Gates and Scott (1986). Measurement locations for the different liners are depicted in Figure 1. The teat load signals are referred to as "TA" and "TB" to denote measurements perpendicular to and within the plane of liner collapse respectively (Figure 1). The magnitude of the effective teat load varied among the liners and among axial and circumferential locations for a given liner.

A comparison between results for a conventional liner and a square bore liner using an artificial teat illustrates the effect of liner geometry (Figure 2). For the conventional liner, the effective teat load was similar within the plane (TB) and perpendicular to the plane of collapse (TA). However, TB of the square bore liner exerted a smaller effective teat load compared with TB of the conventional liner. This is explained by the square bore design - - in the plane of collapse (TB), vacuum can penetrate further up the corners of the square liner, increasing the effect of vacuum on the

measured teat load. This effect was exaggerated in these measurements because contrary to a live teat, the artificial teat did not deform easily into the corners.

Two cows were milked quarter by quarter (8 separate recordings) to measure effective teat load. Two liners were used: (a) a transparent plastic liner (Klear Brand, Milkrite, Johnson Creek, Wisconsin) and (b) a conventional narrow bore liner (De Laval Model 01, Poughkeepsie, New York). For each liner type measurements were made at the top holes depicted in Figures 3 and 4 and at the lower holes (not shown) which were estimated to be lower than the teat during initial placement of the teatcup but within the range of teat stretch during milking.

Each recording for a particular transducer placement site was done on two teats. Thus, a Klear liner was used twice for measurement at the top holes, then the liner was changed for another with holes near the teat end and two more teats were milked. This process was repeated using two standard De Laval liners in the same order. As a consequence, each liner had only two holes to accommodate the transducers.

Measurements in Figures 3 and 4 indicate an initial "small amplitude" portion at the start of milking that occurred at the top holes for the Klear liner (both transducers), the top holes for the De Laval liner at TA only, and at the bottom holes of the De Laval liner. A logical explanation for these small amplitude variations is that during initial high milk flow, the teat is stiffened by the large internal supply of milk. As milk supply diminishes, the teat becomes less rigid and the effective teat load is influenced relatively more by milking vacuum each time the liner opens. This explanation is substantiated by the duration of these small amplitude signals varying from 30 s to 105 s, a typical time for peak milk flow for cows in late lactation with little milk available.

Effective teat load during the liner-open phase varied from 0 to +30 kPa, g (i.e. atmospheric pressure or greater) during the small amplitude portion and varied from -50 to -15 kPa, g during the rest of the measurements. When the liners collapsed, teat load changed only slightly during the small amplitude portion, but generally changed substantially to -10 to +10 kPa, g later in milking. The largest measured effective teat load, approximately 53.5 kPa, g, occurred during liner collapse at TA of the Klear liner (not shown in these Figures). At the top holes for both liners, effective teat load at TA and TB are similar, indicating load axisymmetry. Liner collapse brings the teat load to about atmospheric pressure at both transducers. At lower (distal) portions of the teat perpendicular to the plane of collapse (TA), liner collapse imposes a sufficiently large compressive load on the teat that the effective teat load is greater than atmospheric. The De Laval liner load was approximately axisymmetric, but the Klear liner load was not. The Klear liner resulted in larger values of effective teat load at any liner position primarily due to the smaller bore. Thus, the presence of a liner reduces the effect of vacuum load over the surface of the teat in contact with the liner.

A substantial change in teat load during the course of the entire milking is noted. A minimal circumferential variation (axisymmetry) over much of the teat length was found. These data illustrate the usefulness of the teat load transducer and provide estimates for boundary conditions to predict teat tissue stresses during milking. They are not sufficient to fully characterize many different liner designs currently available. Whether significant differences among liners exist and whether these differences are important from the standpoint of tissue health and milking efficiency remains to be determined.

Reverse Pressure Gradient Across the Teat Canal (Objective 3)

Cross-contamination between liners and reverse transport of milk droplets (potentially contaminated) are the result of temporary pressure differences across the short milk tube which cause air to flow in a direction toward the teat end. The theory of impacts (Thiel, et al, 1969 and Thompson and Pearson, 1979) is the result of such temporary pressure differences. However,

infections have been known to develop when shields which would impede direct droplet impacts were used (Thiel, 1978). Thus, other mechanisms in addition to impacts of direct propulsion through the teat canal must exist.

Susceptibility to new infections has been reported to be greater toward the end of milking (Cousins, et al, 1973) or during liner slip (O'Callaghan, et al, 1976; O'Shea, et al, 1976; O'Shea and O'Callaghan, 1978; O'Shea, et al, 1983; and O'Callaghan, 1988). A reduction in the reverse pressure gradient (RPG) across the short milk tube using partial or restricted liners has shown a reduction in incidence of contamination with partial liner and restricted liner assemblies (Sagi, et al, 1983). Also, several different machine designs which are less prone to cross-contamination and reverse transfer than the conventional unit have shown reductions in the incidence of contamination (Magee, et al, 1983; Noorlander and Heckman, 1985; and Griffin and Grindal, 1981). These research efforts all contributed to stimulating our efforts to establish the importance of reverse pressure gradients in transporting mechanisms through the teat canal.

Determination of pressure gradients across the teat canal requires the measurement of the pressure on both ends of the canal without blocking or interfering with flow through the teat canal. Two pressure transducers (Model PC-350, Millar Instruments Inc., Houston, Texas) were used to make these measurements. One transducer was inserted through a Y connector in the short milk tube and positioned in the liner above the point of collapse, but below the teat end. The teat sinus transducer was inserted through the upper cistern wall using a 10 gauge medicut cannulae with the transducer tip located at the proximal end of the teat canal.

During normal milk flow with no acute air admission, measurements indicated a positive pressure within the teat cistern and a negative pressure in the liner. Towards the end of milking, when milk flow is lower and intramammary pressure decreased, negative pressures developed within the teat sinus corresponding to pulsation. In fact, as the liner opened, a negative pressure was created in the teat sinus and during liner collapse, the pressure in the teat sinus approached atmospheric pressure. This pressure sequence occurred towards the end of milking for all cows. Our work indicates that the vacuum within the teat sinus is primarily a result of extension of vacuum from the milking machine through the open teat canal during the milking phase of each pulsation cycle. Extension of vacuum through the teat canal is largely dependent on the patency of the canal. In our work, the extension of vacuum into the teat sinus ranged from 50 to 90 percent of the vacuum level in the liner. In addition, a secondary factor creating a vacuum within the teat sinus is referred to as the "bellows effect". This "bellows effect" relates to the rapid expansion (movement of teat wall in liner) of the teat which when voided of milk creates a temporary negative pressure in the sinus. The "bellows effect" was determined by sealing the teat canal and measuring pressures within the teat sinus during machine milking.

Manually-induced liner slippage. When liner slips were induced manually by pulling on the teatcup to permit air admission during peak and low milk flows, the sudden change in pressure outside the teat had no effect on pressures measured within the teat sinus. However, when manually induced slips were created during periods of no milk flow (at end of milking), frequent occurrences of reverse pressure gradients (RPGs) were observed (Mahan, 1983; Scott, et al, 1987) in the same teat where slippage was induced. Such a gradient is indicated by a larger negative pressure within the teat sinus than that in the liner. A RPG is created by the sudden lowering of vacuum at the teat end (within liner) which is caused by acute air admission (i.e. liner slippage) and a delayed measured vacuum response in the teat sinus due to the sudden pressure change at the teat end. The highest magnitude of RPG during manual liner slippage was approximately 7 kPa. When manual slippage was induced on the diagonal teat to that of the teat being monitored RPGs were less frequent and lower in magnitude.

Controlled air admissions. Controlled acute air admission of approximately 40, 80, and 160 (l/min) were used to simulate liner slippage experimentally during the following conditions:

1. Acute air admissions in the teatcup diagonal to the teat in which teat sinus measurements were monitored.
2. Acute air admissions within the teatcup of the teat being monitored.
3. Machine removal (a) with vacuum at the teat end and the liner pulsating normally; (b) with vacuum shut-off at the teat end, pulsator deactivated, and the liner in an opened or closed position.

The air admissions were controlled by an electric pulsator governed electrically in time to the air admissions. The pulsator was attached to the short milk tube using a Y connector with an orifice specifically designed for the given rate of air admission. Initiation of the air admission occurred 120 ms into the opening phase of the liner and the duration was for 360 ms. Each acute air admission was conducted at two levels of milking unit reserve (127 and 17 l/min). During acute controlled air admissions, RPGs were frequently created. Such a gradient is illustrated by a larger vacuum level in the teat sinus than in the liner. RPGs have been measured in sixteen teats - - an example illustrating the response from one cow is shown in Figures 5 and 6.

With the methodology of controlled air admissions employed in our studies, RPGs occurred only when sudden air admissions occurred in the liner of the teat being monitored. At the higher vacuum reserve level, RPGs occurred in approximately 50 percent of the cases of an air admission of 160 l/min. while none occurred at the lower air admission levels in the case of high vacuum reserve (Figure 7). At low reserve, RPGs occurred for approximately 75 percent, 5 percent, and 5 percent of the total observations at acute air admissions of 160, 80, and 40 l/min. These results suggest that RPGs occur more frequently during large acute air admissions (160 l/min.) and are dependent on vacuum reserve. During these tests, the magnitude of the RPGs ranged from 1.5 to 7.0 kPa for durations of approximately 20 to 50 ms. No RPGs occurred without a sudden air admission and none were detected with air admissions introduced into the teat diagonal to the teat of measurement.

Effect of machine removal. During machine removal with vacuum at the teat end and the liner pulsating normally, RPGs occurred for approximately 30 percent and 50 percent of the total observations at high and low vacuum reserve levels (Figure 8). During machine removal with liner pulsation or with the pulsator deactivated with the liner either in an open or closed position with liner vacuum shut off at the teat end, we observed similar occurrences of RPGs (20 percent of observations for each) at high vacuum reserve (Figure 9). However, with low vacuum reserve and the same conditions, RPGs occurred with greater frequency and magnitude. However, the high incidence of RPGs in the case of a collapsed liner is strange and, at this point, we do not have an explanation. During machine removal under these conditions the magnitude of RPGs ranged from 1.5 to 15 kPa with durations ranging from 20 to 350 ms. Figure 10 presents a composite of occurrence of RPGs for all experimental results with acute air admission and results for machine removal for both high and low vacuum reserves. Figure 11 shows the results when the vacuum is shut-off to the liner with the pulsator continuing to operate. Figure 12 illustrates the results obtained when the pulsator is shut-off with the liner in a collapsed phase, followed by vacuum shut-off to the liner. In both cases, there is evidence for a reverse pressure gradient at the time of teatcup removal. These findings suggest that the method of machine removal affects the potential occurrence of RPGs.

Bacterial challenge during reverse pressure gradients. A subsequent experiment was designed to determine if organisms could move from outside the teat into the teat cistern during a RPG at the end of milking (no milk flow). Four phages of Staphylococcus aureus and

Staphylococcus aureus (Newbould 305) were used as challenge organisms. The organisms were introduced into the liner through a needle which pierced the liner wall at its base and was attached to an intravenous bottle containing the organisms. Flow rates were set at approximately 10 ml/ min. for each organism. After each test, the pressure transducer in the teat cistern was extracted, exposing the empty cannula through which the teat cistern was flushed with sterile skim milk and aspirated using a canine urinary catheter. Aspirated samples were cultured for respective test organisms.

The results from exposing 8 cows to 34 challenges are shown in Table 1.

TABLE 1.

Challenge	RPGs			
	None	Possible	Present	Total
Absent from Sample	12	4	4	20
Present in Sample	0	3	11	14
Total	12	7	15	34

Relative to Table 1, RPGs are considered as none if the pressure gradient is less than 0.4 kPa, as possible if the pressure gradient is between 0.4 and 1.2 kPa, and as present if the pressure gradient is greater than 1.2 kPa. These results indicate that in no case was an organism obtained from a sample from the teat cistern where no RPG existed. It is also seen that in 14 of 22 challenge cases where an RPG was either possible or present, the organism was obtained from teat cistern samples. Applying a Chi squared test there is statistical significance for the occurrence of the presence of an organism in the teat cistern sample when RPGs are measured to exist. The recovery of these organisms from teat cistern samples during occurrence of RPGs further substantiates that under certain milking conditions, RPGs are likely to be a major factor in organism transfer through the teat canal.

Mechanisms of Pathogen Transport Through The Canal. (Objective 4)

Whereas it has been shown that the milking machine may contribute to the penetration of pathogenic bacteria through the teat canal, it would be wrong to attribute present levels of mastitis exclusively to machine milking. In fact, during the early dry period when machine milking is no longer practiced, dairy cows are more susceptible to mastitis than at any other time of the lactation cycle (Eberhart, 1982). At drying off, Nickerson (1987) states several important changes occur at the teat end which may render a quarter more susceptible to mastitis: (a) the flushing of milk through the teat canal is terminated, (b) milk accumulates and the elevated internal udder pressure induces teat duct dilation allowing easier bacterial penetration, and (c) udder washing and teat dipping are discontinued. Limited research has been done to elucidate the cause of mastitis unrelated to the milking process. While it is clear that the distal end of the teat canal is continually exposed to pathogens, research to analyze mechanisms of bacterial penetration is needed. A pathogen may move through the teat canal not only by direct forces (i.e. RPGs described

previously), but also by indirect propulsion such as diffusion or bacterial multiplication. In addition, diffusion and bacterial growth could act simultaneously and result in pathogenic transport. Thus, we have sought to investigate both analytically and experimentally the transport of mechanisms of pathogenic diffusion and growth into and through the teat canal in dairy cattle.

Bacterial diffusion model. A model for one dimensional diffusion of bacteria through the teat canal predicts the time required for given concentrations of bacteria to diffuse the length of the teat canal. The diffusion model of Delwiche (1981) was modified to allow the movement of bacteria through the teat canal into the teat cistern. Because bacteria adhere to keratin and epithelium in the teat canal, bacterial deposition on teat canal walls has been incorporated into the model (Lissik, 1988). Model equations, the solutions, and results are given in Figures 13 and 14. These Figures show bacterial concentration along the teat canal as a function of position. At short time intervals, there is an initial steep concentration gradient at the distal end of the teat canal which tapers to a 0 gradient at the proximal end. As time increases, the concentration gradient along the teat canal decreases while the overall concentration approaches the initial concentration which is the initial bacterial concentration at the teat end.

The simple diffusion model predicts that bacteria in the teat canal will increase with increasing time. If diffusion is a mechanism of transport of mastitis - causing pathogens in the teat canal, then exposure of the distal end of the teat to a constant source of pathogens should cause a time dependent accumulation of pathogens in the teat canal. This idea was tested by using a preparation with a solution containing 250 mg/ml of digoxin in a sponge was held at the teat end by a finger cot rolled over the teat. Thus, the teat was continually exposed to the tracer and milk samples were obtained from the teat sinus by a teat puncture technique after 4 hours and 10 hours of exposure. Digoxin was detected in all milk samples. The mean value of the digoxin concentration was not different for the exposure periods of 4 and 10 hours. The time of diffusion appears to be less than 4 hours, but experimentation has not developed the minimum exposure time to diffuse the length of the teat canal. However, it should be pointed out that the diffusion coefficient of bacteria is about one thousand times smaller than that of digoxin.

Bacteria growth model. Under optimal conditions, it has been estimated that both Staphylococcus aureus and Streptococcus ahealactiae can grow to a sufficient number in volume to fill the teat canal in 9-14 hours (Lissik, 1988). If the bacterial growth model is coupled to the diffusion equation, we obtain the solution to the model of Figure 15. For a value of $\lambda' = 10$ (low bacterial growth rate), this Figure shows the diffusion profile as a function of length. The bacterial concentration is maximum at the distal end of the teat and decreases toward the proximal end of the teat canal. Because of the exponential growth term in the model, the bacterial concentration at the distal end of the teat canal is larger than the initial concentration at $T > 0$ and as time increases, the concentration of bacteria increases at each point along the length of the teat canal. In addition, the concentration gradient increases along the length of the teat canal and because the rate of bacterial transport by diffusion is proportional to the concentration gradient, pathogen transport into the teat cistern increases with time.

As the rate of bacterial growth increases (a more realistic estimate of λ' is 10,000) the process of diffusion plays an insignificant role in the transport of mastitis-causing pathogens because the growth rate takes over. In fact, under optimal growth conditions, it is estimated that bacteria can grow to a sufficient volume (approximately 2 mm³) to fill the entire canal in approximately 10 hours (Lissik, 1988). As a result, bacterial growth can be considered independent of diffusion at normal population growth rates.

Effects of keratin. The keratin lining in the teat canal is postulated to function as a mechanical barrier to bacterial penetration both by sealing the teat and by its milk repellent properties. As a result, the physical effect of keratin on penetrability of externally applied endotoxin to the teat was tested (Lissik, 1988). The results are: (1) if the keratin lining is left intact in the teat canal,

endotoxin is unable to penetrate into the teat cistern, (2) removal of the keratin lining causes the teat canal to become more susceptible to endotoxin penetration, and (3) removal of the keratin lining causes a significant increase in somatic cell count.

Because removal of keratin leaves the teat more susceptible to bacterial invasions, we conducted a study to determine the rate of regeneration of keratin in the teat canal. If keratin is removed by erosion during milking, it is important to know how long it takes for this protective barrier to regenerate. Based on physical removal of keratin from the teat canal, our measurements show that regeneration is complete in about 24 hours. Fluid shear stresses occurring in the teat canal during milk flow have been estimated by Lissik (1988) but it remains to be determined whether levels of predicted shear stress during milk flow are great enough to cause keratin removal during milk flow.

Effects of Liner Diameter on Teat Morphology (Objective 1)

Teat measurements (morphology, shape, and lesion scores) were carried out in six kibbutz dairy herds, three of which were milked with "narrow" (19-21 mm) bore liners with cluster arm supports and three herds were milked with "wide" (23-25 mm) bore liners without cluster support. Herds were chosen on the basis of identical insemination service, identical milking routine (three times daily at the same hours), similar herd size, same geographic region and the same husbandry routine. In these herds, only cows that were half-sisters in parallel lactations and milked by the two different liner systems were chosen for analysis. Parameters measured were teat dimensions (width and length), teat end lesion score, and clinical status.

Teat dimensions were determined by using a unique photographic system. The developed negatives from the photography process were digitized with a HipadTM Digitizer from Houston Instruments connected to an IBM PC computer for analysis. Teat end lesions were observed immediately after photographing the teat following the end of milking and the degree of lesion was scaled from 0 to 4 on the basis of:

- 0 - Normal
- 1- Smooth, Non-Raised Teat Apex
- 2- Smooth, Ring Around Teat Apex
- 3- Rough, Raised Ring Around Teat Apex
- 4- Very Rough, Ulcerated Tissue Around Teat Apex

Milk samples of 5-10 ml were withdrawn by hand from each teat into test tubes for bacterial analysis. The determination of mastitis was determined by Nagase values and diagnosis based thereupon scaled as:

- 0- Clean
- 1- Non-Pathogenic Infection
- 2- Doubtful Mastitis
- 3- Mastitis
- 4- Severe Mastitis

The working procedure involving the photographing of the teat, the assessment of teat lesions, and acquisition of milk samples were carried out in a manner to limit interference with the milking process. The whole procedure prolonged the milking time for approximately 300 cows by only 20-30 minutes. Thus, the process had only minimal interference with milking routine and led to rather standard conditions for all measurements.

In four herds (two with each liner) the procedure was repeated after 16 months in order to estimate possible differences among the same cows at a later lactation. In addition, a controlled experiment was carried out with six heifers with all the above measurements on all four teats of each heifer every two weeks during a four month period. The first observation began with the first milking of these heifers.

Variations between width and length of the teat in cows of similar characteristics (father, lactation, liner width, and herd) existed. The variation as expressed by standard deviation suggests that it is usually within approximately 10-15 percent for width and rarely greater than 20 percent for teat length. Apart from the individual differences, it was necessary to delay photographing of teats until teat end contractions had stabilized and relaxation had occurred. The effect of liner type on teat dimensions and dimensional factors as a function of lactation number is summarized in Table 2. It is seen that cows milked with the wide liner exhibit teats which are wider and longer for both front and rear. The differences are notable over all lactations. The differences in width and L*W are statistically significant over all cows (Table 3). The difference for teat width is significant over all lactations. When comparing the same cows after 16 months (Table 4) it appears that the wide liner has an effect on teat dimensions for the first and second lactations, but no effect for the third and later lactations. Between lactations three and four, changes are similar for both the narrow and wide bore liners and even a lesser effect with the wide liner. These results are complicated by the larger proportion of cows leaving the herd and, therefore, unavailable for later lactation measurements when milked with the wide liner. From the results of measurements of teat dimensions, there is an indication that the wide liner increases teat dimensions more than the narrow liner. This tends to enlarge the teat volume, leading to speculation that the teat canal may be increased in diameter as well, thus leading to possible reduced resistance to penetration of bacteria.

The results of teat end lesion scores are summarized in Tables 5, 6, and 7 for lactations 1 through 3. For lactations 1 (Table 5) and 3 (Table 7) the results illustrate a trend of higher proportions of severe lesions for those teats milked with the wide liner. However, a statistical analysis did not indicate significant difference for teat lesions across the two liners. For the tests with an interval of 16 months, teat end lesions between the first and second lactation were more severe for those teats milked with the wide liner (Table 8). However, the difference is less notable between lactations 2 and 3 and between 3 and 4.

The proportion of cows with no infected teats (number of teats with an infection rating of 0) is higher in the first and third lactations for cows milked with the narrow liner. In the second lactation, there appears to be no difference between the two liners relative to infections (Table 9). For the second and third lactations, there were no statistical differences between the two sizes of liners relative to number of infections, although there is a tendency for higher numbers of infections with cows milked with the wide liner (Table 10).

From these results, there appears to be an effect on teat morphology dependent on the liner size used for milking. The trend suggests a greater effect on teat width and length and, therefore, teat volume with the wide liner. There also appears to be a negative effect on teat health on the basis of lesser effects on teat lesions and infections with the narrow bore liner. More conclusive results will require additional controlled experiments carried out in the same herd with half-sisters milked from the first day over several lactations utilizing different liner types and including a challenge study for comparison of new infection rates.

DESCRIPTION OF COOPERATION

- Cooperation and collaboration among research leaders in the United States and Israel were excellent throughout the project. However, the sudden and untimely death of Dr. Ram Sagi during the second year of the project required the identification of a new Israeli investigator and the development of a new relationship. Dr. Ephraim Maltz of the Volcani joined the project with a strong interest and commitment to pursue objectives previously outlined by Dr. Sagi.
- Activities throughout the project were initiated and conducted at both U.S. and Israeli locations in accordance with availability of facilities and animals for the specific sub-projects. Sub-projects relating to the application of forces applied by the liner on the teat, measurement and confirmation of reverse pressure gradients across the teat canal, and analysis of pathogenic transport through the

teat canal were best accomplished in terms of personnel and facilities at Cornell University. On the other hand, studies relating to the effects of liner size on teat morphology were more appropriately conducted in Israel with herds in a more field oriented setting.

Clearly, collaboration between U.S. and Israeli researchers on this project has been extensive and has included close interactions. During Dr. Sagi's participation in the project, he typically spent the summers at Cornell University and visited at least one other time during the year, usually in February, to further develop project focus and direction. Dr. Maltz visited Cornell University at the beginning of the third year in order to develop a mutual understanding for the third and last year's research.

EVALUATION OF RESEARCH ACHIEVEMENTS

Research conducted during this project closely followed the original research plan. To meet the basic objective, the work was divided into sub-objectives focusing on liner/teat interactions, quantification of reverse pressure gradients across the teat canal as a mechanism for pathogen transport through the teat canal, theoretical and experimental analysis of mechanisms for transport of mastitis-causing pathogens through the teat canal, and the effects of liner size on teat morphology and teat health. Important achievements are:

- 1) Reverse pressure gradients have been shown to exist under certain milking conditions. Liner slippage or large influx of air flow in the short milk tube at no milk flow at the end of milking has been implicated as possible causes for the development of intramammary infections due to the movement of pathogens through the teat canal. In addition, our findings suggest that the method of machine removal affects the potential occurrence of RPGs. The direct recovery of organisms from the teat sinus following liner slippage, large acute air admissions, and machine removal further substantiates that RPGs are likely to be a primary factor in organism transfer through the teat canal
- 2) Both experimental and analytical results suggest that diffusion may be a mechanism of pathogen transport into the teat canal. However, it is unlikely that the diffusion mechanism alone would support pathogen transport into the teat's cistern during the normal interval between milkings. A more likely mechanism is bacterial growth through the teat canal, combined with the diffusion process.
- 3) Measurements of effective teat loading during machine milking provides a useful means to characterize the effect of basic liner/teat interactions. Our studies have quantitatively characterized the "squeeze" phase of the pulsation cycle and provide evidence that the load applied by the liner actively aids in the redistribution of blood and serum within the teat to relieve teat tissue from the applied vacuum which induces blood and serum pooling within teat tissue which can affect milk removal.
- 4) Results from field studies in six Israeli kibbutzim indicate that the milking system, especially the teatcup assembly can affect teat conformation and possibly the incidence of mastitis. A "wide" liner (23-25 mm) compared with the "narrow" liner (19-21 mm) causes greater increases in teat dimensions and an apparent negative effect on teat health.

These achievements correlate well with the objectives of the original research proposal. Specifically, the experimental verification of reverse pressure gradients across the teat canal is a major contribution to the understanding of the importance of the milking machine under conditions of liner slippage, large acute air admissions, and machine removal to the transport of pathogens through the teat canal, leading to the incidence of mastitis. Also, the understanding of pathogen transport through the teat canal, via a combination of diffusion and bacterial growth, is a major contribution to understanding mechanisms of pathogen transport. These findings provide a better

understanding of mechanisms involved in bacterial penetration through the teat canal and provide the basis for design of machine systems and practices which will reduce the potential for organism transport through the teat canal. The implementation of systems to reduce organism transport on the basis of these findings can have a very significant impact on reducing the incidence of mastitis and, thereby, reduce substantial economic losses as a result of this disease.

LIST OF PUBLICATIONS RESULTING FROM THIS PROJECT

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Lissik, E. A. 1988. Mechanisms of pathogenic transport through the teat canal of a dairy cow. Ph.D. Dissertation, Cornell University, Ithaca, New York. 255 pp.

Lissik, E. A., J. L. Lumley and N. R. Scott. 1989. A model for the fluid mechanics of milk flow through the teat canal of the dairy cow. (Manuscript in preparation)

Lissik, E. A., F. W. Lengemann and N. R. Scott. 1989. Diffusion and growth as mechanisms for pathogen transport into the teat cistern of the dairy cow. (Manuscript in preparation)

Scott, N. R., D. M. Galton, D. J. Aneshansley, E. A. Lissik, R. S. Gates, S. T. Mahan, and P. D. Zehr. 1987. Mechanisms of transfer of organisms through the teat canal. Int'l. Mastitis Symposium, University of Guelph, Ontario, Canada. pp. 66-88.

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Figure 1. Schematic Illustrating Transducer Sites. All Units in mm.

Figure 2. Effective Teat Load Using an Artificial Teat with the IBA Square Bore Liner

Figure 3. Effective Teat Load During Milking. Klear Brand Liner, Top Holes

Figure 4. Effective Teat Load During Milking, De Laval Brand 01 Liner, Top Holes

Figure 5. Simultaneous Measurement of Pressures at External Teat End and Internal Teat Sinus at No Milk Flow. Reverse Pressure Gradients are Shown Following Short Controlled Air Flow Admissions of 160 l/m with a Low Vacuum Reserve of 17 l/m/unit

Figure 6. Expansion of the First Reverse Pressure Gradient Illustrated in Figure 5.

ACUTE AIR ADMISSION

Figure 7. Occurrence of RPGs as a Function of Air Admission for High and Low Vacuum Reserves

MILKING MACHINE REMOVAL (With Vacuum)

Figure 8. Occurrence of RPGs During Machine Removal with Liner Vacuum Applied at High and Low Vacuum Reserves

MILKING MACHINE REMOVAL (w/o Vacuum)

Figure 9. Occurrence of RPGs During Machine Removal with Liner Vacuum Shut-Off with the Liner in an Open, Collapsed, or Pulsating Phase at High and Low Vacuum Reserves.

REVERSE PRESSURE GRADIENT

Figure 10. Occurrence of RPGs During the Application of Acute Air Admissions or Machine Removal Summed Over All Experimental Conditions at High and Low Vacuum Reserves

Amplified scale:

Teat sinus vacuum – Teat end vacuum

Reverse pressure
gradient

Figure 11. A Record of a RPG During Machine Removal with the Vacuum Shut-off to the Liner and with the Pulsator Continuing to Operate at Low Vacuum Reserve of 17 l/m/unit

Figure 11. A Record of a RPG During Machine Removal with the Vacuum Shut-off to the Liner and with the Pulsator Continuing to Operate at Low Vacuum Reserve of 17 l/m/unit

mplified scale:

Teat sinus vacuum - Teat end vacuum

Figure 12. A Record of a RPG During Machine Removal with the Liner in the Collapsed Phase Followed by Vacuum Shut-off to the Liner at a Vacuum Reserve of 127 l/m/unit

Governing Eq.:

$$\frac{\partial^2 \bar{c}}{\partial \bar{z}^2} = \frac{\partial \bar{c}}{\partial \bar{t}}$$

$\bar{c} = \frac{c}{c_0}$: non-dimensional bacterial concentration

Boundary Conditions: $\bar{c}(0, \bar{t}) = 1$

$\bar{z} = \frac{z}{l}$: non-dimensional length

$$\frac{\partial \bar{c}}{\partial \bar{z}}(1, \bar{t}) = 0$$

$\bar{t} = \frac{D t}{l^2}$: non-dimensional time

Initial Condition: $\bar{c}(\bar{z}, 0) = 0$

c_0 : initial bacterial concentration

l : streak canal length

D : diffusion constant

Solution:

$$\bar{c}(\bar{z}, \bar{t}) = 1 - \frac{2}{\pi} \sum_{n=1}^{\infty} \left(\frac{2}{2n-1} \right) \exp \left(- \left(\frac{2n-1}{2} \right)^2 \pi^2 \bar{t} \right) \sin \left(\left(n - \frac{1}{2} \right) \pi \bar{z} \right)$$

Figure 13. A Model for Diffusion of Bacteria Through the Teat Canal of a Dairy Cow

Figure 14. Bacterial Diffusion with Deposition on Teat Canal Walls. The Effect of Removing Bacteria at Different Rates is Shown on the Diffusion Process at a Fixed Time

Governing Eq: $\frac{\partial^2 \bar{c}}{\partial \bar{x}^2} - \lambda \bar{c} = \frac{\partial \bar{c}}{\partial \bar{\theta}}$

$\bar{c} = \frac{c}{C_0}$: non-dimensional bacterial concentration

Boundary Conditions: $\bar{c}(0, \bar{\theta}) = 1$

$\bar{x} = \frac{x}{L}$: non-dimensional length

$\frac{\partial \bar{c}}{\partial \bar{x}}(1, \bar{\theta}) = 0$

Initial Condition: $\bar{c}(\bar{x}, 0) = 0$

$\bar{\theta} = \frac{L^2 t}{D}$: non-dimensional time

λ = non-dimensional bacterial removal rate

C_0 = initial bacteria concentration

L = teat canal length

D = diffusion constant

Solution:

$$\bar{c}(\bar{x}, \bar{\theta}) = \frac{\cosh(\lambda(1-\bar{x}))}{\cosh \lambda} - 4\pi \sum_{n=0}^{\infty} \frac{2n+1}{4\lambda^2 + (2n+1)^2 \pi^2} \sin\left((2n+1)\frac{\pi \bar{x}}{2}\right) \exp\left(-\left(\lambda^2 + \frac{(2n+1)^2 \pi^2}{4}\right)\bar{\theta}\right)$$

Figure 15. Model for Combined Bacterial Diffusion and Growth Through the Teat Canal. Calculations for a Low Bacterial Growth Rate of $\lambda = 10$

Lactation No.	No. of cows	No. of fathers	Front				Rear			
			width(W)	length(L)	L/W	L*W	width(W)	length(L)	L/W	L*W
Narrow liner										
1	89	5	23.58 ±3.43	50.49 ±10.32	2.14 ±0.39	1208 ±370	23.65 ±3.33	45.98 ± 9.12	1.94 ±0.47	1099 ±325
2	61	6	24.59 ±3.56	51.95 ±10.12	2.13 ±0.42	1292 ±370	24.89 ±3.89	46.37 ± 9.90	1.81 ±0.45	1177 ±402
3	47	8	25.65 ±3.48	54.06 ±9.59	2.11 ±0.33	1402 ±386	25.57 ±3.69	46.21 ±9.45	1.81 ±0.32	1198 ±364
4	21	10	26.80 ±3.73	55.93 ±8.57	2.09 ±0.26	1520 ±417	27.00 ±4.24	50.68 ±11.86	1.88 ±0.42	1391 ±468
>4	19	11	27.91 ±4.44	55.08 ±10.12	1.98 ±0.33	1554 ±410	27.88 ±3.44	58.90 ±16.77	2.10 ±0.47	1676 ±634
all	237	13	24.79 ±3.65	52.12 ±9.97	2.12 ±0.37	1310 ±386	24.92 ±3.79	46.92 ±9.97	1.86 ±0.43	1187 ±382
Wide liner										
1	59	4	25.40 ±3.44	53.38 ±14.48	2.09 ±0.39	1392 ±554	25.12 ±3.22	48.76 ±13.40	1.89 ±0.47	1256 ±485
2	66	5	25.87 ±3.35	49.03 ±12.37	1.90 ±0.39	1301 ±394	25.97 ±4.25	48.54 ±11.40	1.87 ±0.54	1313 ±360
3	92	11	27.70 ±3.32	57.20 ±11.44	2.06 ±0.35	1605 ±475	27.80 ±3.38	54.96 ±12.49	1.98 ±0.54	1547 ±476
4	27	8	27.29 ±2.66	55.16 ±8.49	2.02 ±0.31	1513 ±329	27.64 ±3.35	56.49 ±13.47	2.04 ±0.37	1588 ±535
>4	14	8	26.79 ±3.29	53.71 ± 8.65	2.01 ±0.27	1456 ±376	26.98 ±3.61	50.63 ±11.46	1.89 ±0.41	1379 ±418
all	258	13	26.79 ±3.54	54.10 ±12.62	2.03 ±0.37	1479 ±484	26.79 ±3.77	52.67 ±13.75	1.90 ±0.53	1448 ±506

Table 2. Teats dimensions (mm), length: width ratio (L/W) and length width multiplication (L*W) in narrow and wide milking cup liner milked cows.

Lactation No.	Father No.	No. of cows	Front				Rear			
			width(W)	length(L)	L/W	L*W	width(W)	length(L)	L/W	L*W
Narrow liner										
1	1	8	24.84 ±3.53	52.63 ± 8.43	2.13 ±0.25	1328 ±341	25.73 ±3.64	51.92 ±12.40	2.03 ±0.43	1352 ±444
2	1	13	26.50 ±3.02	54.51 ± 5.46	2.06 ±0.26	1450 ±244	29.96 ±3.78	49.45 ± 8.31	1.83 ±0.54	1251 ±546
1	7	22	23.26 ±2.69	51.61 ±11.70	2.21 ±0.38	1220 ±407	22.91 ±2.49	46.15 ±9.19	2.02 ±0.36	1068 ±301
2	7	20	22.70 ±3.40	52.04 ±12.07	2.29 ±0.49	1198 ±407	23.49 ±4.20	47.65 ±10.92	2.03 ±0.26	1157 ±459
1	9	27	25.10 ±3.72	47.83 ± 9.39	1.91 ±0.32	1221 ±371	24.99 ±3.50	44.14 ± 9.04	1.77 ±0.47	1076 ±391
2	9	17	25.56 ±3.75	49.37 ±9.97	1.93 ±0.32	1285 ±406	25.54 ±3.46	43.94 ±8.89	1.72 ±0.52	1133 ±321
1	13	31	22.17 ±2.99	51.20 ±10.13	2.31 ±0.40	1152 ±343	22.40 ±2.95	45.62 ±7.28	2.04 ±0.49	997 ±307
2	13	4	23.08 ±2.15	56.25 ±9.49	2.44 ±0.42	1302 ±259	22.40 ±2.66	41.89 ±7.09	1.87 ±0.28	946 ±225
1	all	88	23.58 ±3.43	50.49 ±10.32	2.14 ±0.39	1208 ±370	23.65 ±3.33	45.98 ± 9.12	1.94 ±0.47	1100 ±325
2	all	54	24.59 ±3.56	51.95 ±10.12	2.13 ±0.42	1207 ±370	24.89 ±3.89	46.37 ±9.90	1.87 ±0.31	1099 ±325

Table 2 - Continued

Lactation No.	Front				Rear			
	width(W)	length(L)	L/W	L*W	width(W)	length(L)	L/W	L*W
1	1%	-	-	6%	5%	-	-	-
2	2%	-	9%	-	1%	-	6%	-
>2	1%	10%	-	5%	2%	6%	-	5%
all	1%	10%	-	1%	1%	7%	-	1%

Table 3. Degree of statistical different significance ($P <$) of dimension parameters and dimension effected factors of teats milked by different milking cup liner.

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Table 4. Effect of 16 month interval on teat dimensions (mm) of cows milked by wide and narrow liner during different lactations.

Degree of teat's end lesion	Number of teats		Number of teats % of n	
	Front	Rear	Front	Rear
Narrow liner n=88				
0	13	12	14.8	13.7
1	49	50	55.7	56.8
2	22	23	25.0	26.1
3	4	3	4.5	3.4
4	-	-	-	-
Wide liner n=57				
0	5	6	8.8	10.5
1	25	27	43.9	47.4
2	17	19	29.8	33.3
3	9	5	15.8	8.8
4	1	-	1.7	-

Table 5. Teat's end lesion score of 1st lactation cows milked by different milking cup liner (n=number of cows scored).

Degree of teat's end lesion	Number of teats		Number of teats % of n	
	Front	Rear	Front	Rear
Narrow liner n=65				
0	3	3	4.6	4.6
1	22	20	33.9	30.8
2	34	25	52.3	38.4
3	6	17	9.2	26.2
4	-	-	-	-
Wide liner n=61				
0	5	6	8.2	9.8
1	29	31	47.5	50.8
2	21	19	34.4	31.2
3	6	5	9.9	8.2
4	-	-	-	-

Table 6. Teat's end lesion score of 2nd lactation cows milked by different milking cup liner (n=number of cows scored).

Degree of teat's end lesion	Number of teats		Number of teats % of n	
	Front	Rear	Front	Rear
Narrow liner n=46				
0	2	-	4.3	-
1	16	19	34.8	41.3
2	19	16	41.3	34.8
3	9	10	19.6	21.7
4	-	1	-	2.2
Wide liner n=114				
0	1	3	0.9	2.6
1	29	26	25.4	22.8
2	43	42	37.8	36.9
3	33	36	28.9	31.6
4	8	7	7.0	6.1

Table 7. Teat's end lesion score of 3rd lactation cows milked by different milking cup liner (n=number of cows scored).

Liner type	n	Front		Rear	
		unchanged (± 1 score units)	changed ($\pm 2,3$ score units)	unchanged (± 1 score units)	changed ($\pm 2,3$ score units)
First score Lactation 1, second score Lactation 2					
Narrow	15	80.0	20.0	86.7	13.3
Wide	9	66.7	33.3	66.7	33.3
First score Lactation 2, second score Lactation 3					
Narrow	10	70.0	30.0	70.0	30.0
Wide	8	87.5	12.5	62.5	37.5
First score Lactation 3, second score Lactation 4					
Narrow	5	100	-	80.0	20.0
Wide	17	76.5	23.5	70.5	29.5

Table 8. Effect of 16 months interval on teat ends lesion of cows milked by wide and narrow liner during different lactations (n = No. of cows compared)

No of teats infected	1st lactation		2nd lactation		3rd lactation	
	No of cows with infected teats	No of cows with infected teats as % cows scored	No of cows with infected teats	No of cows with infected teats as % cows scored	No of cows with infected teats	No of cows with infected teats as % cows scored
Narrow liner						
0	38	42.7	21	34.4	20	42.5
1	30	33.7	25	41.0	13	27.6
2	12	13.5	12	19.7	6	12.8
3	7	7.9	3	4.9	6	12.8
4	2	2.2	-	-	2	4.3
Wide liner						
0	17	28.3	22	32.3	19	17.0
1	16	26.7	25	36.8	44	39.3
2	21	35.0	15	22.1	31	27.7
3	5	8.3	3	4.4	17	15.2
4	1	1.7	3	4.4	1	0.8

Table 9. Number of infected teats in cows milked by different milking cup liner.

Type of infection	Number of teats infected				Number of teats infected a% of cows scored			
	Front		Rear		Front		Rear	
	right	left	right	left	right	left	right	left
Narrow liner								
0	47	48	45	46	77.1	78.7	73.8	75.4
1	10	8	9	5	16.4	13.1	14.8	8.2
2	2	2	2	4	3.3	3.3	3.3	6.5
3	1	1	4	2	1.6	1.6	6.5	3.3
4	1	2	1	4	1.6	3.3	1.6	6.5
Wide liner								
0	49	50	48	49	72.1	73.5	70.6	72.0
1	11	10	12	11	16.2	14.7	17.6	16.2
2	5	5	8	7	7.3	7.4	11.8	10.3
3	1	3	-	1	1.5	4.4	--	1.5
4	2	-	-	-	2.9	-	--	-

Table 10. Type of infection found in teats of 2nd lactation cows milked by different milking cups liner.

APPENDICES