Journal of Dairy Research (1995) **62** 395–404 *Printed in Great Britain*

Physical characteristics of the bovine teat canal and their influence on susceptibility to streptococcal infection

BY S. JANE LACY-HULBERT* AND J. ERIC HILLERTON
Institute for Animal Health, Compton Laboratory, Compton, Newbury RG16 0NN, UK

(*Received 25 April 1994 and accepted for publication 29 November 1994*)

SUMMARY. Physical characteristics of the bovine teat canal were examined for their influence on susceptibility to intramammary infection. All quarters of 18 cows were inoculated with 2×10^5 cfu *Streptococcus agalactiae* (Trial 1) and 20 cows with 10^5 cfu *Str. uberis* (Trial 2) 3–4 mm into the teat canal every 3 d for 12 d. Incidence of quarter infection was similar for both pathogens, 30/72 (42%) in Trial 1 and 32/80 (40%) in Trial 2. Logistic regression analysis showed that probability of infection by *Str. agalactiae* increased significantly with an increase in quarter peak flow rate ($P < 0{\cdot}05$) whereas probability of infection increased for *Str. uberis* with a decrease in teat canal length ($P < 0{\cdot}05$). A significantly higher ($P < 0{\cdot}001$) incidence of infection by *Str. uberis* was observed in quarters that contained a low wet weight ($< 1{\cdot}8$ mg) of removable keratin compared with those that contained $> 1{\cdot}8$ mg keratin, but there was no correlation between weight of keratin and length of the teat canal. Infections by *Str. uberis* took significantly less ($P < 0{\cdot}05$) time to show a rise in somatic cell count above $7{\cdot}5 \times 10^5$ cells/ml than *Str. agalactiae*. The results provide evidence that these pathogens use different mechanisms to pass through the teat canal.

Dodd & Neave (1951) identified a direct relationship between rate of milking and the incidence of naturally occurring mastitic infections by staphylococci and *Streptococcus agalactiae* during lactation and by *Str. uberis* during the dry period. Grindal & Hillerton (1991) showed, under conditions of experimental challenge with *Str. agalactiae*, an enhanced risk of mastitis among fast flow rate quarters when milked without pulsation or with a high frequency of impacts. The rate of milk flow is controlled directly by the size of the milking vacuum and the diameter of the teat canal orifice (Baxter *et al.* 1950).

Histological studies by McDonald (1971) revealed that teat canals of quarters susceptible to infection by *Staphylococcus aureus*, *Staph. epidermidis* and coliform bacteria were greater in diameter and had a thinner keratinous canal lining than quarters that did not become infected. Murphy (1959) demonstrated that partial removal of the keratin temporarily reduced natural resistance of the canal to invasion by *Str. agalactiae*. McDonald (1975*a*) suggested that the shorter teat canal was more susceptible to penetration by pathogens, although Murphy & Stuart (1955) failed to show any relationship between length of the teat canal and resistance to infection by *Str. agalactiae*. Grega & Szarek (1985) observed, however, that Polish Black and White Lowland cows had shorter (9·21 mm) and wider (0·89 mm) teat

* Present address: Dairying Research Corporation Ltd, Private Bag 3123, Hamilton, New Zealand.

canals and a higher incidence of mastitis than Polish Reds, which had longer (12·05) and narrower (0·78 mm) canals. Chandler *et al.* (1969) suggested that the mesh-like structure of keratin might impede progress of cocci through the canal. Any foreign material or bacteria that becomes attached to the keratin would be flushed from the canal by milk flow following cell desquamation (Williams & Mein, 1985).

The risk of infection by *Str. uberis*, an environmental pathogen, is greatest during early lactation (Cousins *et al.* 1980) but little is known about the mechanism by which this pathogen passes through the teat canal of the lactating animal (Bramley, 1984). Previous examinations of the teat canal have concentrated on infections by *Staph. aureus* or by *Str. agalactiae*, two pathogens capable of colonizing the teat canal (Du Preez, 1985). Various properties of the canal, principally length, diameter and weight of keratin occluding the lumen should influence the passage of pathogens through the canal. The relationships between such properties and intramammary infection by *Str. agalactiae* and by *Str. uberis* were investigated and compared so that the respective mechanisms of bacterial penetration could be hypothesized.

MATERIALS AND METHODS

Experimental design

All four quarters of 18 Holstein–Friesian cows were exposed to *Str. agalactiae* in Trial 1 and all quarters of a separate group of 20 Holstein–Friesian cows were exposed to *Str. uberis* in Trial 2. The animals were of mixed parity and in mid lactation. All quarters were shown to be free from intramammary infection at the start of the 12 d experimental periods by bacterial examination of two quarter fore milk samples (International Dairy Federation, 1981) and had no known history of infection by the pathogens that were to be inoculated experimentally. The cows were selected to include a wide range of quarter peak flow rates (0·32–2·22 kg/min). Cows were assigned randomly to one of two milking stalls and milked in a specific order twice a day, starting at 05.30 and 15.00. All animals were housed in a covered cubicle yard for the duration of both experiments.

All quarters were challenged by inoculation of the teat canal following afternoon milking on days 1, 4, 7 and 10 of a 12 d experimental period, using the Newbould inoculator (Newbould & Neave, 1965). This model was used previously by Grindal *et al.* (1991). Each canal was inoculated with 2 μl of a bacterial suspension, to a depth of 4 mm in Trial 1 or 3 mm in Trial 2. Preferred depth of inoculation was 3 mm but the appropriate inoculators were not available for Trial 1. The difference between an inoculation at 3 or 4 mm is not thought to have a significant effect on the subsequent full penetration of bacteria (Prasad & Newbould, 1968; Bramley & Higgs, 1974).

Quarter fore milk samples were collected aseptically (International Dairy Federation, 1981) from all quarters at the first and tenth milking before the experiment and at the sixth, seventh and ninth milking after the last bacterial inoculation for bacterial examination. Aseptic sampling of fore milk was avoided during the experimental period so as not to disturb bacterial populations within the teat canal. Additional duplicate fore milk samples were taken if there was severe clotting of the milk. Fore milk samples were collected non-aseptically for determination of somatic cell count (SCC) using a Coulter counter at morning milking prior to each bacterial inoculation.

Intramammary infection was diagnosed if, in two consecutive quarter fore milk samples, there was an elevated SCC ($> 7{\cdot}5 \times 10^5$ cells/ml) in combination with clots in the milk and/or isolation of > 50 cfu/ml of the inoculated pathogen. Quarters

showing severe clinical signs were treated at three successive milkings by intramammary infusion of 200 mg cloxacillin and 75 mg ampicillin (Kloxerate Plus MC; Duphar Vet Ltd, Southampton SO30 4QH). The same treatment was applied to all quarters irrespective of infection status ~ 7 d after the last bacterial challenge.

Preparation of bacterial suspension

In Trial 1 inocula contained 2×10^5 cfu each of *Str. agalactiae* 221/22 (NCDO 1520) and *Str. dysgalactiae* CE 127 (NCDO 2043). In Trial 2 inocula contained ~ 10^5 cfu/ml of *Str. uberis* O140J. Bacteria were grown separately in 10 ml sterile skim milk (Oxoid, Basingstoke RG24 0PW) at 37 °C for 18–20 h. Fresh suspensions at the appropriate dilution were prepared in sterile skim milk for each day of inoculation.

Measurement of teat canal properties

Peak flow rate of individual quarters was recorded electronically by averaging the flow rate over the period of maximum flow (Butler *et al.* 1990) at five morning milkings during the pre-experimental period. The teat canal length of all quarters was measured immediately prior to afternoon milking using a cannula, modified according to Grindal *et al.* (1991). The average of three measurements was used for each teat canal, obtained 28 d after the experimental periods to avoid any influence on the study of bacterial infection.

Collection of keratin

The amount of keratin present within each teat canal was estimated by collection within the eye of a size 14G tapestry needle (Henry Milward & Sons, Studley B80 7AS). This collection technique is considered to remove ~ 70% of the total removable keratin present in the teat canal (Bright *et al.* 1990). Needles and keratin samples were transported within stoppered 5 ml polypropylene test tubes to avoid contamination and loss of moisture or keratin. Tubes and needles were weighed before and within 1 h of reaming and the wet weight of keratin determined by the difference in weights, measured to $\pm 0{\cdot}1$ mg. Keratin was harvested before afternoon milking on three occasions at 2 d intervals, subsequent to full recovery from infection.

Milking procedure

Before milking, fore milk was drawn from each quarter into a strip cup for examination. Teats were washed and dried thoroughly before attachment of the cluster. Each quarter was milked separately through a low level quarter recorder jar system (Butler *et al.* 1990) using shielded liners. Milking conditions were as described by Grindal *et al.* (1991). A computerized data recording system allowed automatic measurement of quarter yield, flow rate and milking time (Butler *et al.* 1990). Cluster removal was activated when the milk flow from the last quarter to finish milking fell below 0·25 kg/min. During the period of bacterial exposure teatcups were immersed in hot water (80 °C for 5–10 s) and allowed to drain before milking the next cow so as to restrict cross contamination of bacteria between cows.

Statistical analysis

Simple associations between teat canal properties and incidence of intramammary infection were examined using Student's t test and χ^2 within Minitab (Minitab Inc., Pennsylvania, PA 16801, USA). More detailed logistic regression analyses were performed using Genstat (Rothamsted Experimental Station, Harpenden AL5 2JQ).

Linear logistic regression models were constructed to correlate the sum of infections experienced by each cow with her average teat canal results and to determine the relative importance of each teat canal characteristic in determining cow susceptibility to infection.

RESULTS

Thirty of the 72 (42%) inoculated quarters became infected in Trial 1; 70% of these quarters displayed clinical signs with 15 quarters requiring antibiotic therapy prior to the end of the infection period. All infected quarters expressed > 50 cfu/ml of *Str. agalactiae* into the fore milk on more than one occasion. Only one quarter excreted both *Str. agalactiae* and *Str. dysgalactiae*, suggesting that the inoculation challenge method was unsuitable for *Str. dysgalactiae*. The influence of this organism was therefore ignored during subsequent analyses. A similar rate of infection was observed in the second trial with 32 of the 80 quarters (40%) becoming infected with *Str. uberis* and 30 quarters requiring immediate antibiotic therapy.

The mean peak flow rate of quarters that became infected with *Str. agalactiae* ($1{\cdot}24 \pm 0{\cdot}09$ kg/min, mean ± SE) was significantly higher ($P < 0{\cdot}05$) than the mean peak flow rate of the uninfected quarters ($1{\cdot}02 \pm 0{\cdot}05$ kg/min, $t = 2{\cdot}3$ for 70 d). The mean teat canal length of the same infected quarters ($11{\cdot}3 \pm 0{\cdot}43$ mm) was not significantly ($P = 0{\cdot}11$) lower than that of the uninfected quarters ($12{\cdot}2 \pm 0{\cdot}32$ mm). No other obvious differences were observed between the averaged teat canal characteristics of the infected and the uninfected quarters in either trial, so the distribution of infections across the different quarters was examined. Quarters were divided into four groups for each teat canal property using the median, upper and lower quartile values as the divisors and the incidence of infection in each group compared (Table 1).

Peak flow rate

Some 67% of the highest flow rate quarters (> 1·4 kg/min) became infected by *Str. agalactiae* whilst only 28% of the lowest flow rate quarters (< 0·8 kg/min) became infected. The incidence of infection with a peak flow rate > 1·2 kg/min, a value chosen arbitrarily by Grindal *et al.* (1991), was 63% (15/24) and was significantly higher ($P < 0{\cdot}05$) than the incidence of 31% (15/48) observed for quarters with a peak flow rate < 1·2 kg/min.

In Trial 2 no relationship between incidence of *Str. uberis* infection and rate of peak flow was apparent (Table 1). However the incidence of infection above the arbitrary peak flow rate of 1·2 kg/min was 56% (15/27) and differed significantly ($P < 0{\cdot}05$) from the incidence of 21% (17/53) observed for quarters with a peak flow rate < 1·2 kg/min.

Length of the teat canal

There was an apparent inverse relationship between the length of the teat canal (Table 1) and proportion of infected quarters in Trial 1, but this was not significant. No such relationship could be seen in Trial 2.

Weight of keratin

Although the average weight of reamed keratin and the likelihood of *Str. agalactiae* infection appeared related (Table 1) the incidence of infection did not differ significantly between each category. In Trial 2, those quarters that yielded a low weight of keratin (< 1·8 mg) experienced a significantly higher ($P < 0{\cdot}05$) rate of

Table 1. *Proportion of quarters exposed to high bacterial challenge for 12 d that became infected within each category of 'peak flow rate', 'teat canal length' and 'keratin weight'*

(Quarters were divided into four groups using the median and upper and lower quartiles for each property; infection is presented as quarters infected/quarters exposed, with the percentage infected in parentheses)

	Trial 1: *Str. agalactiae*		Trial 2: *Str. uberis*	
	Quarters	Quarters infected	Quarters	Quarters infected
Quarter peak flow rate, kg/min	< 0·80	5/18 (28%)	< 0·75	8/19 (42%)
	0·80–1·10	7/18 (39%)	0·75–0·94	6/21 (29%)
	1·11–1·40	6/18 (33%)	0·95–1·27	9/20 (45%)
	> 1·40	12/18 (67%)	> 1·27	9/20 (45%)
Teat canal length, mm	< 11	8/15 (53%)	< 10	11/27 (41%)
	11–12	8/17 (47%)	10–11·5	8/16 (50%)
	12·1–13	8/18 (44%)	11·6–13·5	8/20 (40%)
	> 13	6/22 (27%)	> 13·5	5/17 (29%)
Wt of keratin, mg/quarter	< 2·34	7/20 (35%)	< 1·2	9/20 (45%)
	2·34–3·33	7/18 (39%)	1·2–1·83	13/20 (65%)
	3·34–4·33	7/18 (39%)	1·84–2·43	4/20 (20%)
	> 4·33	9/16 (56%)	> 2·43	6/20 (30%)
Overall rate of infection		30/72 (42%)		32/80 (40%)

infection (22/40 or 55%) by *Str. uberis* than those quarters that yielded a high weight of keratin (> 1·8 mg; 10/40 or 25%).

Statistical analysis

Owing to the very low within-cow variation of peak flow rate and teat canal length (results not shown), the observations for each quarter were not strictly independent, thereby compromising the use of χ^2 analysis for these two characteristics. For more detailed analyses values were averaged across the four quarters of a cow and related to her sum of infections.

Linear logistic regression analysis. Regression models were constructed to describe the influence of each teat canal characteristic on the probability of infection within each trial. The relative importance of each characteristic was determined by removing each factor in turn from the model and recalculating the residual deviance. The observed change in the residual deviance was used to determine a significance value.

In Trial 1 the residual deviance (22·3) of the three-factor model was greater than the 14 residual degrees of freedom, indicating overdispersion of the residual error. This suggested that an infection in one quarter increased the probability of an infection occurring in another quarter. This finding may have reflected the use of teat canal values averaged across a cow, since the peak flow rate and teat canal length of quarters within a cow are not strictly independent. F values for each factor of the model are recorded in Table 2. Repeated removal of the least significant factor gave a two-factor and a single-factor model and the F values for the constituent parts are also recorded (Table 2).

In Trial 2 the residual degrees of freedom (16) and the residual deviance (18·9) were similar, indicating very little overdispersion of the error. F values were calculated for the components of three-, two- and single-factor models (Table 2).

In the two-factor model for Trial 1, peak flow rate was the significant ($P < 0·05$) determinant of probability of infection (p) whilst teat canal length was a non-significant secondary factor ($P = 0·07$). Further reduction of the model to a single

Table 2. *Statistics of the three teat canal characteristics used to construct linear regression models describing probability of infection by* Str. agalactiae *or by* Str. uberis

Trial 1: *Str. agalactiae*		Trial 2: *Str. uberis*	
Three-factor model	F†	Three-factor model	F‡
Peak flow rate	6·00*	Peak flow rate	3·30§
Teat canal length	2·37	Teat canal length	5·77*
Keratin wt	1·09	Keratin wt	0·08
Two-factor model		Two-factor model	
Peak flow rate	6·40*	Peak flow rate	3·24‖
Teat canal length	3·74§	Teat canal length	5·74*
Single-factor model		Single factor model	
Peak flow rate	4·19	Teat canal length	2·65

† For 1 and 14 df.
‡ For 1 and 16 df.
Probabilities for F values: * $P < 0{\cdot}05$, § $P = 0{\cdot}07$, ‖ $P = 0{\cdot}09$.

Table 3. *Odds ratios with 95% confidence intervals associated with a 0·1 kg/min change in peak flow rate and 1 mm change in teat canal length for both experiments*

Trial	Factor	Coefficient (c)	Odds ratio (e^{-c})	95% Confidence interval
1	Peak flow rate	2·31	1·26	1·03–1·54
1	Teat canal length	−0·39	0·68	0·47–0·97
2	Peak flow rate	1·83	1·14	0·96–1·50
2	Teat canal length	−0·40	0·67	0·46–0·98

factor, containing peak flow rate and a constant, substantially reduced rather than increased the significance of the regression, thereby suggesting that both factors were necessary to the model describing susceptibility to *Str. agalactiae* infection. The relationship was

$$\ln(p/(1-p)) = 2{\cdot}31(\text{peak flow rate}) - 0{\cdot}39(\text{teat canal length}) + 1{\cdot}65.$$

In the two-factor model for Trial 2, teat canal length was the significant factor and peak flow rate the secondary factor ($P = 0{\cdot}09$). The relationship was

$$\ln(p/(1-p)) = 1{\cdot}83(\text{peak flow rate}) - 0{\cdot}40(\text{teat canal length}) + 3{\cdot}12.$$

Since these were both linear relationships, the size and sign of the coefficients indicated the degree and direction respectively of the influence of each teat canal property on the probability of infection. For both experiments an increase in peak flow rate and/or a reduction in teat canal length increased the probability of infection.

Analyses of the odds ratios. Each equation coefficient represents the natural logarithm of the odds ratio, a value representing the risk or odds of an infection occurring in any quarter. Using the SE values, 95% confidence intervals were calculated for each odds ratio (Table 3). Confidence intervals of each factor were reasonably narrow and did not on the whole overlap or include unity, at which point the odds of an infection would remain unchanged for a unit change of a factor. This indicated a highly consistent effect of each property on the probability of infection. Confidence intervals for teat canal length were surprisingly similar across the trials, suggesting that, from the point of view of the cow, teat canal length had a protective function which was common to both groups of cows.

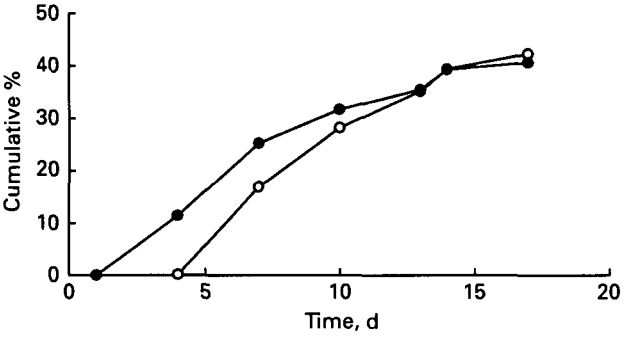

Fig. 1. Time course of clinical appearance of infections caused by ○, *Str. agalactiae*; ●, *Str. uberis*, indicated by the cumulative percentage of quarters exhibiting an elevated somatic cell count ($> 7{\cdot}5 \times 10^5$ cells/ml). Bacterial inoculation took place on days 1, 4, 7 and 10; for details, see text.

Timing and appearance of infections

The infections due to *Str. uberis* occurred sooner after inoculation than the *Str. agalactiae* infections despite the use of a smaller inoculum for *Str. uberis* and inoculation at a shallower depth. Diagnosing an infection as an SCC $> 7{\cdot}5 \times 10^5$ cells/ml the average time taken for each infected quarter to show an elevated SCC was significantly ($P < 0{\cdot}05$) longer for *Str. agalactiae* infections (10 d) than for *Str. uberis* infections (7 d) (Fig. 1).

DISCUSSION

An infection rate of 90–100 % is usually observed following an infusion of the teat sinus with either *Str. agalactiae* (Murphy & Stuart, 1953) or *Str. uberis* (Hill, 1988), suggesting little involvement of intramammary immune defences with susceptibility to infection. The lower incidence observed in experiments involving teat duct inoculation allows some assessment of the teat duct defences and their importance in the prevention of intramammary infection. When quarters were exposed to either *Str. agalactiae* or *Str. uberis*, a similar incidence of infection was observed overall.

Certain characteristics of the teat canal differed significantly between those quarters that developed an infection or resisted infection, suggesting the importance of these characteristics in the control of bacterial passage through the canal. In both trials quarters with a peak flow rate $> 1{\cdot}2$ kg/min experienced significantly more infections than those with a lower peak flow rate. Regression analysis indicated a significant ($P < 0{\cdot}05$) and positive relationship between peak flow rate and susceptibility to *Str. agalactiae* infection but not for *Str. uberis* infection, although the model did suggest that flow rate was a contributory factor. The absence of very high peak flow rate quarters in the group of animals used for Trial 2 may have compromised detection of this effect. In both experiments a negative or inverse relationship was shown between the length of the teat canal and probability of infection. For *Str. agalactiae* this factor was secondary to that of flow rate, failing to achieve statistical significance at the 5 % level, whereas for *Str. uberis* teat canal length was the more important (and significant, $P < 0{\cdot}05$) factor.

The differing relative importance of peak flow rate and canal length for the two pathogens suggests that mechanisms used by *Str. agalactiae* to penetrate the teat canal are influenced more by the diameter of the teat canal lumen, as reflected by the peak flow rate, than by the canal length. Conversely, mechanisms used by *Str. uberis* are influenced more by the length of the teat canal than by the diameter.

Examination of the odds ratios, which showed similar confidence intervals across the experiments for both peak flow rate and teat canal length, suggest that, with regard to a particular quarter, these factors are an important but non-specific part of the primary defences of the teat canal.

Dodd & Neave (1951) suggested that certain bacteria could more readily gain entrance to the gland through the larger or slacker teat canal commonly associated with the faster milking cow. The increased risk of infection for the quarter with a short teat canal length has been suggested often but rarely observed when using less sophisticated methods of data collection and analysis (Murphy & Stuart, 1955; McDonald, 1975*b*).

Determination of the relevance of keratin was complicated by the significantly lower weight of keratin obtained from teats in the second trial. Nevertheless a positive although non-significant relationship was observed between incidence of infection by *Str. agalactiae* and the amount of removable keratin present in each quarter. In contrast the incidence of *Str. uberis* infections was significantly higher among quarters from which less than the median of 1·83 mg of keratin could be removed, suggesting that the likelihood of infection was greater in those quarters that contained a thinner layer of keratin. McDonald (1971) also found a higher incidence of naturally occurring staphylococcal and coliform infections among quarters with a thinner, more particulate keratin layer.

The significant difference in time taken for infections to develop provides further evidence that the two pathogens use different mechanisms to pass through the teat canal. Infections due to *Str. uberis* occurred, on average, 2 d earlier than those caused by *Str. agalactiae*. Since an immunological response to a teat sinus infusion of either pathogen occurs typically within 24 h of infusion (Mackie *et al.* 1983) and detection of invading pathogens is thought to occur only after bacteria have passed the Furstenberg rosette at the apex of the canal (Newbould & Neave, 1965; Collins *et al.* 1986), these results suggest that *Str. uberis* were able to pass through the teat canal in a shorter length of time than *Str. agalactiae*.

It has been reported that *Str. agalactiae* can colonize the teat canal prior to infection of the gland (Du Preez, 1985). The teat canal containing a large weight of keratin would provide a larger surface area available for bacterial colonization but the relationship between the weight of keratin and the dimensions of the teat canal are not yet known. Strong adhesion of bacteria to keratin, particularly to the less mature, unsloughed keratin cells, would enable the successful pathogen to withstand the flushing forces of milk flow. Since *Str. agalactiae* has no hyaluronic acid extracellular capsule (Mamo *et al.* 1986), high cell surface hydrophobicity would encourage adherence of this organism to the lipid-coated keratin cells (Williams & Mein, 1985). Protein-mediated adherence of *Str. agalactiae* to bovine mammary gland epithelial cells due to extracellular fimbrae has also been reported (Bramley & Hogben, 1982). In contrast this particular strain of *Str. uberis* has a hyaluronic capsule (Leigh *et al.* 1990) which would impart a negative charge to the cell surface of the bacterium and favour its retention within an aqueous environment, such as the milk residues within a recently milked teat canal.

Little is known about the mechanism used by *Str. uberis* to penetrate through the canal and colonization of keratin has not been reported (Bramley, 1984). The shorter time taken for *Str. uberis* to pass through the canal compared with *Str. agalactiae* and the greater importance of canal length over peak flow rate observed in the logistic regression analysis would suggest that the penetration mechanism is quicker than that for *Str. agalactiae*. This might involve transient retrograde movement of milk

(Johnston, 1938; Scott *et al.* 1987) or milk components (Craven, 1985). Until now investigations of the penetration mechanisms have concentrated on organisms capable of colonizing the teat canal, *Str. agalactiae* (Grindal *et al.* 1991) and *Staph. aureus* (Bramley *et al.* 1979). More work is required to examine the interaction between keratin and *Str. uberis*.

Despite these differences, both flow rate and teat canal length significantly influenced the likelihood of infection by both pathogens under normal milking conditions. These factors are reasonably consistent within a single animal and yet a number of quarters remained uninfected and were not necessarily the longest teat canals or lowest peak flow rate quarters of a particular animal. Removal of keratin under experimental conditions can significantly affect the likelihood of infection (Capuco *et al.* 1992). Investigations of the lipid and protein components of keratin have identified antibacterial factors (Adams & Rickard, 1963; Hibbitt *et al.* 1969) but few of the findings have been substantiated by later work (Hogan *et al.* 1987, 1988). Most intramammary pathogens are able to grow and multiply in a medium where teat canal keratin provides the only source of nutrients (Murdough *et al.* 1991). Natural resistance to infection by a quarter may therefore include other, unidentified properties of the teat canal such as the rate of keratin turnover or chemical interactions between keratin and milk residues e.g. the xanthine oxidase–lactoperoxidase system suggested by Collins *et al.* (1988).

We are grateful to Mr N. J. Adley, Dr M. C. Butler, Mr R. J. Grindal, Mr S. C. Howell, Mr R. T. Staker, Dr R. M. Teverson and Mr A. W. Walton for advice and technical assistance and to Mrs E. Allan for help with the statistical analysis.

REFERENCES

Adams, E. W. & Rickard, C. G. 1963 The antistreptococcic activity of bovine teat canal keratin. *American Journal of Veterinary Research* **24** 122–135

Baxter, E. S., Clarke, P. M., Dodd, F. H. & Foot, A. S. 1950 Factors affecting the rate of machine milking. *Journal of Dairy Research* **17** 117–127

Bramley, A. J. 1984 *Streptococcus uberis* udder infection – a major barrier to reducing mastitis incidence. *British Veterinary Journal* **140** 328–335

Bramley, A. J. & Higgs, T. M. 1974 Growth of bacteria through the streak canal of unmilked cows. *International Dairy Congress* **19** New Delhi, **1E** p. 134

Bramley, A. J. & Hogben, E. M. 1982 The adhesion of human and bovine isolates of *Streptococcus agalactiae* (group B) to bovine mammary gland epithelial cells. *Journal of Comparative Pathology* **92** 131–137

Bramley, A. J., King, J. S., Higgs, T. M. & Neave, F. K. 1979 Colonization of the bovine teat duct following inoculation with *Staphylococcus aureus* and *Escherichia coli*. *British Veterinary Journal* **135** 149–162

Bright, S. A., Bitman, J., Capuco, A. V., Wood, D. L. & Miller, R. H. 1990 Methods of collection and lipid composition of teat canal keratin in dry and lactating cows. *Journal of Dairy Science* **73** 98–106

Butler, M. C., Allen, C. J. & Hillerton, J. E. 1990 Methods of measuring and calculating milking performance of cows. *Journal of Agricultural Engineering Research* **46** 245–257

Capuco, A. V., Bright, S. A., Pankey, J. W., Wood, D. L., Miller, R. H. & Bitman, J. 1992 Increased susceptibility to intramammary infection following removal of teat canal keratin. *Journal of Dairy Science* **75** 2126–2130

Chandler, R. L., Lepper, A. W. D. & Wilcox, J. 1969 Ultrastructural observations on the bovine teat duct. *Journal of Comparative Pathology* **79** 315–319

Collins, R. A., Parsons, K. R. & Bland, A. P. 1986 Antibody-containing cells and specialised epithelial cells in the bovine teat. *Research in Veterinary Science* **41** 50–55

Collins, R. A., Parsons, K. R., Field, T. R. & Bramley, A. J. 1988 Histochemical localization and possible antibacterial role of xanthine oxidase in the bovine mammary gland. *Journal of Dairy Research* **55** 25–32

Cousins, C. L., Higgs, T. M., Jackson, E. R., Neave, F. K. & Dodd, F. H. 1980 Susceptibility of the bovine udder to bacterial infection in the dry period. *Journal of Dairy Research* **47** 11–18

Craven, N. 1985 Do rising fat-globules assist microbial invasion via the teat duct between milkings? *Kieler Milchwirtschaftliche Forschungsberichte* **37** 554–558

Dodd, F. H. & Neave, F. K. 1951 Machine milking rate and mastitis. *Journal of Dairy Research* **18** 240–245

Du Preez, J. H. 1985 Teat canal infections. *Kieler Milchwirtschaftliche Forschungsberichte* **37** 267–273

GREGA, T. & SZAREK, J. 1985 Relationship of teat canal size to milkability and udder health in three breeds. *Zeszyty Naukowe Akademii Rolniczej w Krakowie* no. 191 *Zootechnika* **23** 3–11

GRINDAL, R. J. & HILLERTON, J. E. 1991 Influence of milk flow rate on new intramammary infection in dairy cows. *Journal of Dairy Research* **58** 263–268

GRINDAL, R. J., WALTON, A. W. & HILLERTON, J. E. 1991 Influence of milk flow rate and streak canal length on new intramammary infection in dairy cows. *Journal of Dairy Research* **58** 383–388

HIBBITT, K. G., COLE, C. B. & REITER, B. 1969 Antimicrobial proteins isolated from the teat canal of the cow. *Journal of General Microbiology* **56** 365–371

HILL, A. W. 1988 Pathogenicity of two strains of *Streptococcus uberis* infused into lactating and nonlactating bovine mammary glands. *Research in Veterinary Science* **45** 400–404

HOGAN, J. S., PANKEY, J. W. & DUTHIE, A. H. 1987 Growth inhibition of mastitis pathogens by long-chain fatty acids. *Journal of Dairy Science* **70** 927–934

HOGAN, J. S., SMITH, K. L., TODHUNTER, D. A. & SCHOENBERGER, P. S. 1988 Growth responses of environmental mastitis pathogens to long-chain fatty acids. *Journal of Dairy Science* **71** 245–249

INTERNATIONAL DAIRY FEDERATION 1981 Laboratory methods for use in mastitis work. Brussels: IDF (*International Dairy Federation Bulletin* no. 132)

JOHNSTON, T. 1938 Anatomical and experimental study of the teat of the cow with particular reference to streptococcal mastitis. *Journal of Comparative Pathology and Therapeutics* **51** 69–77

LEIGH, J. A., FIELD, T. R. & WILLIAMS, M. R. 1990 Two strains of *Streptococcus uberis*, of differing ability to cause clinical mastitis, differ in their ability to resist some host defence factors. *Research in Veterinary Science* **49** 85–87

MCDONALD, J. S. 1971 Microscopic observations of teat canals from susceptible and resistant bovine mammary glands: a preliminary report. *Proceedings of the VIth International Conference on Cattle Diseases, pp.* 97–103

MCDONALD, J. S. 1975*a* Radiographic method for anatomic study of the teat canal: changes between milking periods. *American Journal of Veterinary Research* **36** 1241–42

MCDONALD, J. S. 1975*b* Radiographic method for anatomic study of the teat canal: characteristics related to resistance to new intramammary infection during lactation and the early dry period. *Cornell Veterinarian* **65** 492–499

MACKIE, D. P., POLLOCK, D. A., MENEELY, D. J. & LOGAN, E. F. 1983 Clinical features of consecutive intramammary infections with *Streptococcus agalactiae* in vaccinated and non-vaccinated heifers. *Veterinary Record* **112** 472–476

MAMO, W., FRÖMAN, G. & WADSTRÖM, T. 1986 Bacterial adhesion as a virulence factor in bovine mastitis: possible role of cell surface hydrophobicity, capsule, fibronectin, fibrinogen and collagen binding. *Proceedings of the Symposium on Mastitis Control and Hygienic Production of Milk*, Espoo, Finland, pp. 77–87 (Ed. M. Sandholm)

MURDOUGH, P. A., MARTUS, N. S., MAZZOLA, G. J., SALAMUN, D. M., SCUDDER, P. J., URBANO, M. A. & PANKEY, J. W. 1991 In vitro growth studies of mastitis pathogens on teat canal keratin. *Journal of Dairy Science* **74** (Suppl. 1) 204 Abstr. P186

MURPHY, J. M. 1959 The effect of certain mild stresses to the bovine teat canal on infection with *Streptococcus agalactiae*. *Cornell Veterinarian* **49** 411–421

MURPHY, J. M. & STUART, O. M. 1953 Some results of the application of *Streptococcus agalactiae* (Cornell 48 strain) to the bovine teat canal by means of the Hadley-Wisconsin swab technique. *Cornell Veterinarian* **43** 465–480

MURPHY, J. M. & STUART, O. M. 1955 Teat canal length in the bovine and its relation to susceptibility to swab-induced infection with *Streptococcus agalactiae*. *Cornell Veterinarian* **45** 112–122

NEWBOULD, F. H. S. & NEAVE, F. K. 1965 The effect of inoculating the bovine teat duct with small numbers of *Staphylococcus aureus*. *Journal of Dairy Research* **32** 171–179

PRASAD, L. B. M. & NEWBOULD, F. H. S. 1968 Inoculation of the bovine teat duct with *Staphylococcus aureus*: the relationship of teat duct length, milk yield and milking rate to development of intramammary infection. *Canadian Veterinary Journal* **9** (5) 107–115

SCOTT, N. R., GALTON, D. M., ANESHANSLEY, D. J., LISSIK, E. A., GATES, R. S., MAHAN, S. T. & ZEHR, P. D. 1987 Mechanisms of transfer of organisms through the teat canal. *Proceedings of the International Mastitis Symposium*, Canada, pp. 66–78

WILLIAMS, D. M. & MEIN, G. A. 1985 The role of machine milking in the invasion of mastitis organisms and implications for maintaining low infection rates. *Kieler Milchwirtschaftliche Forschungsberichte* **37** 415–425