Susceptibility of the bovine udder to bacterial infection in the dry period

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SUMMARY. The teats of 18 cows were externally exposed to infection with mastitis organisms by dipping them daily for 1 or 2 weeks in a mixed suspension of *Streptococcus dysgalactiae* and *Str. uberis* at the start, the middle or the end of the dry period. The teat sinuses of quarters which remained uninfected after external exposure at the beginning or the middle of the dry period were then infused with the same strains of streptococci. Of the 9 quarters infected following external exposure 8 occurred in animals exposed at the start and one in the middle of the dry period. Thirty-six new infections occurred after infusion of bacteria into the teat sinuses of 38 quarters. In a further trial with 10 cows, *Staphylococcus aureus* and *Str. zooepidemicus* were inoculated into the distal 3 mm of the streak canals of 5 cows immediately after drying-off and into those of 5 cows which had been dry for 28 weeks. Animals were slaughtered 48 h later and infection determined by teat puncture. Five infections occurred in cows which were at the start of the dry period and only one in the cows dry for 28 weeks. It is suggested that these differences in the rates of new infection between the early dry period and a very extended dry period are due to differences in the ease with which bacteria can penetrate the teat canal. At the later stage bacterial growth through the teat canal appeared to be inhibited.

The unmilked udder is particularly susceptible to infection in the 3 weeks following drying-off (Neave, Dodd & Henriques, 1950). Indeed, rates of new infection during the dry period exceed those of lactation, which may be due to the absence of the flushing out effect of regular milking (Neave *et al.* 1968; Thomas *et al.* 1972). However, this could not account for the large difference in the rate of new infection between the early part and the later part of the dry period. This difference may be due to the higher level of exposure to bacteria at the beginning of the dry period, i.e. bacteria remaining on the teat skin from the preceding lactation period. Alternatively, progressive changes in the composition of the udder secretion during the dry period (Wheelock *et al.* 1967; Smith, Wheelock & Dodd, 1967*b*) may influence the establishment of infection after bacterial penetration of the streak canal. It is also possible that changes within the streak canal, such as the formation of a natural seal, may make bacterial penetration more difficult in later stages of the dry period. The aim of the experiments described in this paper was to investigate further the changes in susceptibility to new infection during the dry period. Two experiments were carried out; the first was designed to expose the udders of dairy cows to similar numbers of pathogenic bacteria either at the beginning, the middle or towards the end of the dry period and to measure the resulting numbers of new infections. The second

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experiment investigated directly whether, during the dry periods changes occur in the ease with which bacteria can penetrate the streak canal.

EXPERIMENTAL

Expt 1

Eighteen cows of various lactation ages were used for the experiment. Each cow was dried-off exactly 8 weeks before the estimated date of calving and allocated to 1 of 3 treatments. The cows were exposed to infection either in early, mid or late dry period. Exposure was at only one stage of the dry period because an infection in the early dry period and its subsequent therapy might have influenced a cow's susceptibility to a further infection in the later stages of the dry period. The 3 experimental treatments were as follows:

Treatment I. The udders of 3 cows were exposed externally to bacteria from d 3 to 9 of the dry period and those of another 3 from d 3 to 16.

Treatment II. The udders of 3 cows were exposed externally to bacteria from d 22 to 35 of the dry period and those of another 3 from d 29 to 35.

Treatment III. The udders of 3 cows were exposed externally to bacteria from d 39 to 52 of the dry period and those of another 3 from d 50 to 56.

The teats were exposed to pathogens by immersion in a bacterial suspension on each prescribed day; for the remainder of the dry period the teats were dipped each day in a disinfectant solution (4% available chlorine).

Quarters allocated to treatments I and II which were not infected by external exposure were then challenged by infusion of bacteria into the teat sinus.

Experimental routine

From milk samples taken late in lactation, one week before drying-off and after the last milking of the lactation, it was established that all quarters of all cows were free from intramammary infection with staphylococci and streptococci.

Before each of the external exposure periods began, 2 quarters of each udder were sampled for bacteriological examination. The other 2 quarters were not sampled, in order to determine whether sampling, which could 'break' any naturally occurring 'seal' of the streak canal, influenced susceptibility to new infection. Three days after each period of external exposure to bacteria was completed, fore-milk samples were taken from each quarter on 2 successive days. When the results of the bacteriological examination of these samples were known the uninfected quarters were infused with bacteria. These infusions were restricted to the 12 cows receiving treatments I or II and were therefore given approximately 3 and 6 weeks after drying-off. Seventy-two and 96 h after infusion the infused quarters were sampled to determine whether a new intramammary infection had occurred. All quarters which had become infected, either as a result of external or internal exposure to bacteria, then received antibiotic therapy. An intramammary infusion of 100000 units of both procaine penicillin G and dihydrostreptomycin (Streptopen QR, Glaxo Laboratories Ltd, Greenford, Middlesex, England) was given on 3 occasions at 24-h intervals. Fore-milk samples were taken from these quarters on d 7 and 13 after the last infusion of antibiotic to determine whether the infection had been eliminated.

On the day of calving 2 fore-milk samples were taken from each quarter and further samples were taken on d 5 and 10 after calving.

The udders were palpated daily throughout the dry period to detect any abnormalities such as hardness, tenderness or swelling. If a quarter showed severe clinical

signs at any time, 2 fore-milk samples were taken and antibiotic therapy was given immediately.

If the udder was dirty when fore-milk samples were needed or quarters required to be infused, it was washed with warm running water and dried with a paper towel before disinfection and sampling.

Preparation of bacterial cultures

For external exposure to bacteria all teats were dipped daily during the exposure period in a suspension of bacteria in litmus milk. The suspension contained 2 strains of *Str. dysgalactiae* (CE 127; 401/10) and 2 strains of *Str. uberis* (265; HE 134). The 4 strains of bacteria were grown separately in litmus milk for 16–18 h at 37 °C, and diluted in litmus milk to contain approximately 8×10^6 colony forming units (cfu/ml) of each strain in the suspension. This was distributed in bottles of 100 ml and stored at 2–4 °C. On each day the contents of one bottle were poured into a plastic beaker and the teats were contaminated by dipping them in the beaker which was pushed against the udder to form a seal and then shaken. A fresh suspension was prepared weekly.

For infusions, bacterial suspensions were prepared which contained approximately 20, 80, 2×10^4 or 8×10^4 cfu/0·25 ml litmus milk. The teats of the quarters to be infused were cleaned thoroughly with 70% (v/v) ethanol and 0·25 ml of suspension was infused through the streak canal into the teat sinus by means of a syringe and smooth-ended needle. Twenty cfu were infused into 11 quarters, 80 cfu into 8 quarters, 2×10^4 cfu into 10 quarters and 8×10^4 cfu into 9 quarters. There were 2 levels of infusion within cows, either 20 and 2×10^4 cfu or 80 and 8×10^4 cfu.

Diagnosis of intramammary infection

The fore-milk samples were taken after scrubbing the ends of the teats with 70% ethanol. All fore-milk samples, except those taken when a quarter showed severe clinical signs during the period of external exposure, were taken during the period when the teats were being dipped daily in disinfectant, and 0·05 ml of the sample was plated on aesculin ox-blood agar (ABA). The plates were examined after 48-h incubation at 37 °C.

An infection was assumed to have occurred if large numbers of bacteria were present in at least 2 successive fore-milk samples. In most cases the secretion of quarters diagnosed as infected was distinctly different macroscopically from that of uninfected quarters.

Expt 2

A mixed suspension of bacteria in 0·002 ml skim-milk containing 1×10^5 cfu of haemolytic *Staph. aureus* NCDO 1499 and *Str. zooepidemicus* NIRD 0065 grown as for expt 1 was inoculated into the distal 3 mm of streak canals of (i) 5 first-calf heifers immediately after drying-off following lactations of 3–6 weeks only and (ii) 5 cows which had been unmilked for 28 weeks. The quarters used in this trial were free of infection and showed no evidence of colonization of the teat duct by staphylococci or streptococci. Infected or damaged quarters were omitted. Quarters of the 5 cows unmilked for 28 weeks were not sampled before teat duct inoculation, to avoid removing material in the sinus or teat duct. The inoculations were infused into the streak canals either 12, 24 or 48 h before slaughter, using the inoculator described by Newbould & Neave (1965).

Immediately after slaughter and thorough cleaning of the teat skin with 70%

Table 1. *The number of new infections resulting from external exposure to, or infusion with* Streptococcus dysgalactiae *and* Str. uberis *at the beginning of the dry period (expt 1)*

Cow no.	Lactation no.	Qtr	Period of external exposure, d after drying off	New infections following external exposure to bacteria	New infections following infusion of bacteria	No. cfu infused
11	4	RF	3–9	11	—	—
		RH*		0	0	80
		LF*		0	III	8×10^4
		LH		11	—	—
490	2	RF*	3–9	0	II, III	8×10^4
		RH		0	II, III	8×10^4
		LF		III	—	—
		LH*		0	II, III	80
500	2	RF*	3–9	0	III	80
		RH		0	II	80
		LF		0	II	8×10^4
		LH*		0	II, III	8×10^4
306	6	RF	3–16	0	II	20
		RH*		0	II	20
		LF*		0	II, III	2×10^4
		LH		0	II, III	2×10^4
402	4	RF	3–16	III	—	—
		RH*		III	—	—
		LF*		0	II	20
		LH		0	II	20
539	1	RF*	3–16	III	—	—
		RH		III	—	—
		LF		III	—	—
		LH*		0	II, III	2×10^4

cfu, Colony forming units.
II, *Str. dysgalactiae.*
III, *Str. uberis.*
— Quarter not exposed to, or infused with, bacteria.
* Quarter sampled immediately before the period of external exposure.

ethanol, a milk sample was taken from the teat sinus through the wall of the teat using a syringe and needle (Murphy & Stuart, 1954). A negative swab of the skin at the site of sampling, together with isolation of the specific bacteria in the milk samples was taken to be indicative of an intramammary infection. The teats were then cut from the udder and deep frozen. Transverse sections (approximately 2 mm thick) of the streak canal were cut under strict aseptic conditions from the frozen teats and swabs of the different sections were plated immediately on ABA.

RESULTS

The results of expt 1 are summarized in Tables 1, 2 and 3. None of the quarters sampled immediately before the start of an exposure period was found to be infected. Of the 9 infections that followed external exposure to bacteria, 8 occurred in the 6 cows receiving the exposure at the beginning of the dry period (treatment I), one occurred in the 6 cows exposed in the middle of the dry period (treatment II) and none followed exposure at the end of the dry period (treatment III). Seven of the

Table 2. *The number of new infections resulting from external exposure to, or infusion with,* Streptococcus dysgalactiae *and* Str. uberis *in the middle of the dry period (expt 1)*

Cow no.	Lactation no.	Qtr	Period of external exposure, d after drying-off	New infections following external exposure to bacteria	New infections following infusion of bacteria	No. cfu infused
390	4	RF*	29–35	0	II, III	80
		RH		0	II, III	80
		LF		0	II, III	8×10^4
		LH*		0	II, III	8×10^4
521	1	RF*	29–35	0	III	2×10^4
		RH		—	—	—
		LF		0	II, III	20
		LH*		0	II, III	20
561	1	RF		0	II	8×10^4
		RH		0	II, III	8×10^4
		LF*		0	II, III	80
		LH		0	II, III	80
455	3	RF	22–35	0	0	2×10^4
		RH*		0	II	2×10^4
		LF*		0	II	20
		LH		0	II	20
523	1	RF*	22–35	0	II, III	20
		RH		0	II, III	20
		LF		0	II, III	2×10^4
		LH*		0	II, III	2×10^4
533	1	RF	22–35	0	III	2×10^4
		RH*		0	III	2×10^4
		LF*		0	II	20
		LH		III	—	—

cfu, Colony forming units.
II, *Str. dysgalactiae.*
III, *Str. uberis.*
—, Quarter not exposed to, or infused with, bacteria.
* Quarter sampled immediately before the period of external exposure.

infections were with *Str. uberis* and 2 were with *Str. dysgalactiae*. Six of the 9 infections were in cows exposed for 2-week periods rather than 1 week, and 4 of the 6 cows receiving treatment I became infected. Only 2 of the new infections occurred in quarters sampled before an exposure period, compared with 7 in the unsampled quarters.

Two streptococcal infections occurred before cows were exposed either internally or externally to the bacterial suspension. Both of these were first detected by clinical signs, one 8 d and the other 19 d after the last milking of lactation.

More infections occurred after inoculation of the teat sinuses of those quarters which were not infected as a result of external exposure to bacteria. Of the 38 quarters which did not become infected as a result of external exposure to bacteria, 36 became infected after inoculation of the teat sinus. Five of these infections were with *Str. uberis*, 11 with *Str. dysgalactiae* and 20 were mixed infections with both *Str. uberis* and *Str. dysgalactiae*. As all but 2 of the infused quarters became infected it was not possible to demonstrate differences in susceptibility to infection during the early or middle stages of the dry period or between different numbers of bacteria infused.

Table 3. *The number of new infections resulting from external exposure to* Streptococcus dysgalactiae *and* Str. uberis *towards the end of the dry period (expt 1)*

Cow no.	Lactation no.	Qtr	Period of external exposure, d after drying-off	New infections from external exposure
161	7	RF	50–56	0
		RH*		0
		LF*		0
		LH		0
541	1	RF*	50–56	0
		RH		0
		LF		0
		LH*		0
587	1	RF*	50–56	0
		RH		0
		LF		0
		LH*		0
232	7	RF	39–52	0
		RH*		0
		LF*		0
		LH		0
487	2	RF*	39–52	0
		RH		0
		LF		0
		LH*		0
509	2	RF*	39–52	0
		RH		0
		LF		0
		LH*		0

* Quarter sampled immediately before the period of external exposure.

Table 4. *Numbers of quarters infected with* Staphylococcus aureus *and* Streptococcus zooepidemicus *in animals after inoculation (inoc) of the teat duct 12–48 h before slaughter*

	5 Cows dry for 28 weeks				5 Heifers immediately after drying-off			
	Hours between inoc and slaughter				Hours between inoc and slaughter			
	12	24	48	Totals	12	24	48	Totals
No. of teat ducts inoculated	5	5	5	15	4	4	4	12
Intramammary infection (teat wall puncture sample positive)*	1	0	0	1	0	1	4	5
Bacteria not recovered from streak canal	2	1	2	5	0	0	0	0
Bacteria recovered from site of inoculation only	2	4	3	9	3	3	0	6
Bacteria recovered from site of inoculation and to rosette of Furstenburg	1	0	0	1	1	1	4	6

* *Staph. aureus* and *Str. zooepidemicus* recovered.

All but 2 of the infected quarters yielded secretion with many clots while none of the other uninfected quarters of the cow showed comparable symptoms except one infused quarter that was temporarily infected with *Str. dysgalactiae*.

In expt 2, the inoculation of 16 quarters of 5 heifers either 48, 24 or 12 h before drying-off resulted in 5 intramammary infections. Four of these occurred when the infusion was 48 h before drying-off, one when it was 24 h and none occurred with the 12-h interval (Table 4). Nearly all infected quarters had mixed staphylococcal and streptococcal infections and large numbers of these organisms were isolated from the whole length of the streak canal. In the quarters which had not become infected it was always possible to isolate both organisms from the site of inoculation.

In the cows which had not been milked for 28 weeks, only one intramammary infection occurred. This was with *Str. dysgalactiae* although *Staph. aureus* had grown within 1 mm of Furstenburg's rosette. No bacteria were isolated from 5 of the inoculated streak canals and in the remaining 9 the bacteria were isolated only from the site of the inoculation.

DISCUSSION

The results of the first experiment demonstrate that when udder quarters are exposed externally to similar numbers of bacteria, infections are much more likely to occur at the beginning of the dry period than in the later stages. These results are consistent with the observations of Neave *et al.* (1950) who measured naturally occurring infections. However, the results of a previous experiment (Thomas *et al.* 1972) indicate that the greater susceptibility in the early dry period lasts for at least 2 weeks.

It would appear that the higher rate of new infection in the early dry period is mainly because at this time bacteria are able to penetrate the streak canal to the teat sinus more easily, rather than there being a change in susceptibility of the quarters. Even very small numbers of bacteria infused through the streak canal into the teat sinus at the beginning or the middle of the dry period resulted in new intramammary infections. Either the bacteria can multiply more easily in the secretion within the streak canal at the beginning of the dry period, or the conditions which occur soon after regular milking ceases may favour movement of bacteria within the streak canal by physical means. Thomas *et al.* (1972) were unable to demonstrate that intramammary pressure, leakage of milk, or composition of the secretion influenced infection rate in the early dry period.

The resistance to infection in the later stages of the dry period may be due to the plug of sebum-like material of the streak canal keratin which prevents either the growth of bacteria or their physical movement. However, if this hypothesis were correct more new infections would have been expected in the 2 quarters of each cow which were sampled immediately before external exposure to bacteria began. In fact more new infections occurred in the unsampled quarters. Further experiments are necessary to determine why the resistance of the streak canal to the penetration of bacteria changes during the dry period.

Most of the infections which occurred following external exposure to bacteria were with *Str. uberis* although the bacterial suspension contained approximately equal numbers of both *Str. uberis* and *Str. dysgalactiae*. An increase in the number of infections with *Str. uberis* during the dry period was also noted by Neave *et al.* (1950) and Smith *et al.* (1967*a*). This may be due to the greater ability of *Str. uberis* to colonize the external orifice of the streak canal at this time, thereby increasing the

exposure to this organism, or to relatively more favourable conditions within the udder for the growth of this pathogen.

The second experiment indicates that bacteria can penetrate the streak canal more readily in the early dry period. In animals in which milking ceased immediately before inoculation of bacteria into the streak canal, the bacteria were able to multiply within the streak canal and, if the time interval was long enough, penetrate to the teat sinus. However, in cows which had been unmilked for approximately 28 weeks there was evidence of growth from the site of inoculation in only one quarter and in some instances the bacteria were not able to survive at the site of inoculation. This suggests the presence of a bacterial inhibitor within the streak canal at later stages of the dry period which prevents the multiplication of bacteria within the streak canal and subsequent penetration to the teat sinus.

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