**E. COLI MASTITIS - THE PAST, THE PRESENT AND THE FUTURE**

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**SUMMARY**

1. The challenge of *Escherichia coli* udder infection will remain so long as cows produce faeces.

2. *E. coli* mastitis is a disease of wet environments. Infection is via the teat end, in a variety of situations.

3. The outcome of a case of *E. coli* udder infection is determined by the inflammatory response of the cow rather than the pathogenicity of the invading strain.

4. There is a case for not treating mild cases of *E. coli* mastitis with antibiotics if an on-farm test for *E. coli* endotoxin in mastitic milk proves fast and reliable. Perhaps there is a place for the farm laboratory, similar to the already accepted farm workshop.

5. *E. coli* udder infection towards the end of the dry period, with infected quarters presenting as clinical cases of mastitis in the following lactation, is an important aspect of the problem.

6. Vaccination should protect against acute clinical *E. coli* mastitis.

7. We need to find some way of effectively separating cows from their excreta, although *E. coli* mastitis problems are not restricted to herds with obvious bad hygiene.

“*In science you can say what you like, so long as you can prove it*”.

Absolute truth is something of an abstract concept. In the field of mastitis there are facts established by experimental work completed under controlled conditions with statistically valid results repeated independently by different workers, sometimes in different countries and in different years. Opinions, usually based on experience, are very common and all should be considered. They can sometimes be tested by simple logic or scientific investigation. The degree of certainty with which an opinion is expressed is not necessarily a measure of its accuracy.

For practical purposes there is no limit to knowledge. A vast amount has been published on *E. coli* and on mastitis during this century. Handling knowledge is a major problem and much might be achieved by correct application of what we already know, rather than further expensive research.
PRE-HISTORY

The mammals inherited the earth from the dinosaurs about 65 million years ago: humans and what can be described as cattle first appeared in the Pliocene Era, between 0.5 and 2 million years ago: the earliest evidence to date of domestication is around 6400 BC (1). *E. coli*, being found in the colon of all mammals, will also have evolved millions of years ago. The long association is likely to be a safe one under natural conditions (although pathological strains of *E. coli* which cause enteritis have evolved) and it appears that *E. coli* mastitis results from accidental infection (2). Strains of *E. coli* recovered from cases of mastitis are the same as those in the cow’s environment, and would presumably attempt growth in the same way in milk in a jug as in a cow’s udder.

HISTORY

*E. coli* (3), was first recorded in bovine mastitic milk in 1896 (4). Kitt (5) reproduced the disease by “lightly sticking” (sic) *E. coli* to teat orifices. There are at least 35 references to *E. coli* bovine mastitis before 1935 (6). Murphy and Hanson (7) described a three year investigation into coliform udder infection recording 79 infections in the 120 cow herd; 31 were first detected in colostrum and a proportion of known infected quarters developed clinical mastitis early in the following lactation. 59.4% of total isolates were *Aerobacter aerogenes* and 14.5% *E. coli*. Schalm and Wood (8) described acute “coliform” (*A. aerogenes*) mastitis in a California dairy herd in sawdust yards following penicillin “blitz” treatment to eradicate *Streptococcus agalactiae*. They postulated that resulting low milk cell counts allowed udder invasion of *A. aerogenes* from sawdust bedding and their consequent experiments tended to confirm this (9).

Up until the late 1960s, the majority of cases of subclinical and clinical mastitis in the UK were caused by *Staphylococcus aureus* and *Str. agalactiae*. The source of both was cow’s udders, and infection spread at milking time. What later came to be called the “five point plan” was initiated in the UK and copied in many other countries and proved successful in controlling these two infections. No other pathogen has taken their place in cow’s udders. The five point plan has not been successful in controlling coliform mastitis. There has been a very substantial rise in the incidence of *E. coli* mastitis since 1960, and it is the most common cause of fatal mastitis (10,11).

LOW CELL COUNTS

It appears impossible to initiate *E. coli* mastitis in a quarter with an established *Str. agalactiae* sub-clinical infection but easy to do so in a low cell count quarter, suggesting that constitutive defences are ineffective (9). (The concept of a pathogen implies an organism which can partially overcome or evade the body’s constitutive defences). The concept of the udder which is “on its guard” responding quickly to invasion by bacteria, compared to a quiescent udder has been proposed (12). The question of whether this could occur in the national herd has been raised on numerous occasions. There is certainly evidence that low cell counts can predispose to herd *E. coli* mastitis problem (13,14); but a large number of what used to be called low cell count herds have no serious *E. coli* mastitis problem.

BREEDING
There has been a general selection of cows for faster milking rate, which is associated with mastitis susceptibility, and is inherited (15). Long term it is likely that many diseases will be controlled by breeding disease resistant animals. Higher yielding cows in four UK dairy herds tended to develop *E. coli* mastitis, but there was no correlation with yield *per se* (16).

**MILKING PROCEDURES**

*E. coli* can enter cow’s udders in a variety of situations (17). *E. coli* does not colonise healthy teat skin (18). The teat duct is apparently the major barrier to intramammary infection (19).

When three groups of cows’ teat ends were contaminated with *E. coli* broth cultures (109 cfu’s/ml) before, during and after milking, for three weeks: 23 of 120 quarters (11/30 cows) became infected, 10 developing clinical mastitis (20). There was no difference in infection incidence between the three groups.

A significant incidence of fatal coliform mastitis in suckler cows was recorded in Northern Ireland in a 1992 survey of cow mortality (10). These cases cannot be blamed on the milking machine!

**POST MILKING TEAT DISINFECTION**

This is part of the five point plan mastitis control plan. Effective post milking teat disinfection is thought to sterilise the proximal teat canal. There are organisms normally present there as commensals (e.g. *Campylobacter bovis*) and a number of workers have postulated that these organisms could have a protective effect. For a bacterium to become established on a surface, it must first displace the established flora. There is no evidence that *E. coli* establishes in the teat canal prior to invasion of the udder. There is some evidence that post milking teat disinfection with an iodophor increased the rate of *E. coli* mastitis (21). A more comprehensive Dutch survey (22) calculated a decrease in clinical *E. coli* mastitis of 71% if post milking iodophor teat dipping was discontinued.

**DIAGNOSIS**

Although acute clinical *E. coli* mastitis can be diagnosed fairly confidently by the practising veterinary surgeon, and any peracute mastitis in a low cell count herd is likely to be environmental in origin, it is impossible to be 100% certain without laboratory confirmation. Usually this is based on isolation of *E. coli* in pure culture from mastitic milk. A high proportion of *E. coli* mastitis, particularly cases in the latter half of lactation are not acute, and the organism may have disappeared from the udder before clinical mastitis is detected. Even in some cases of peracute mastitis, numbers of organisms can be very low or absent. In these latter cases, the isolation of only three organisms from one drop of milk is significant. Aliquots of this milk can be incubated in nutrient broth and sub-cultured. If *E. coli* is isolated in pure culture, it is considered to have caused the mastitis. When organisms cannot be recovered, the sample could be tested for the presence of endotoxin. In fact this is the ideal test - *E. coli* mastitis is caused by endotoxin, not merely by the presence of the organism in the udder. The Limulus Amoebocyte Lysate Test can be used to detect endotoxin in mastitic milk (27).
Contamination of samples is a major hazard in confirming *E. coli* mastitis because the organism is normally present in the cow’s environment. It can be particularly complicating when detecting sub-clinically infected carrier cows. The author developed a system of collecting samples from suspect quarters after the cows had been milked, when the teat canal would have been thoroughly flushed out. In some cases, milk had to be incubated at 37°C, or incubated in nutrient broth and sub-cultured. If *E. coli* was recovered on culture on a number of consecutive occasions, it was taken to indicate persistent infection.

**THERAPY**

Mastitis is caused by endotoxin released from the walls of disintegrating *E. coli*. The majority of cases of *E. coli* mastitis, particularly in the second half of the lactation, are mild and cases self cure. Mild cases can return to complete normality. There is considerable evidence that antibiotics do not significantly alter the course of peracute *E. coli* mastitis. However, it would be a very brave veterinary surgeon who would not administer antibiotics, and there are also publications supporting the use of antibiotics. Administration of non antibiotic therapy may depend on the clinical assessment by the attending veterinary surgeon - i.e. cows with different presenting signs may need different therapy. It is likely that antibiotics are wasted in the treatment of mild *E. coli* mastitis. In Norway, mastitic milk is tested on farm for *E. coli* endotoxin. If a positive reaction is found then no antibiotics are given. There could well be a future for this approach in this country. The test does cost money, and takes time. The danger of not treating mild coccal mastitis is that it will become established, and the herd bulk milk cell count will rise even if cases remain mild.

**THE DRY PERIOD**

The conclusion from English experimental work in the 1970s was that the dry udder was generally refractory to *E. coli* infection (23). A number of workers in the USA starting in 1943 (7), described the appearance of *E. coli* infection during the late dry period. Culturing milk samples from all newly calved cows for coliforms, and treating positive quarters with antibiotics to prevent clinical episodes in the subsequent lactation became a recommendation in veterinary textbooks by the early 1980s (c.f. 24); also sealing teats of “easy milkers” in the last third of the dry period. Only 6% of “coliform” clinical mastitis cases found during lactation occurred in quarters sub-clinically infected at calving in five UK herds (25). Very extensive work in Somerset confirmed the general eradication of existing *E. coli* udder infections at drying off and the appearance of new infections detected from two weeks prior to calving (26). Of all enterobacterial mastitis occurring in the first 100 days of lactation, 52% arose in quarters previously infected during the dry period, with the same strains of bacteria. Current recommendations include the application of teat seals in the final weeks of the dry period, rather than culture and therapy.

It is extremely important to avoid introduction of bacteria into the udder with dry cow therapy. Some organisms, including *E. coli*, but notably Pseudomonad spp., are resistant to some of the antibiotics used in dry cow therapy and will cause serious clinical mastitis.

**FEEDING/DIET**

Anecdotal observations suggested that low fibre, high protein silage, predisposed to *E. coli* mastitis in Norway (28). Controlled trials (29) did not confirm that a high protein diet predisposed to mastitis, although perhaps this was because it was balanced with carbohydrates.
E. coli mastitis often follows herd diarrhoea problems (30). Widely different faecal E. coli counts in cows on different diets were found in a herd investigation (31), the newly calved animals receiving the most concentrates having the highest counts and the most E. coli mastitis. A comprehensive study (32) revealed only slightly higher faecal E. coli counts in high yielding cows. There appears to be no correlation of late pregnancy diet with environmental mastitis (33). E. coli mastitis correlated with feeding lush spring and autumn grass in a zero grazed herd (34).

Fatty liver does not influence the severity of E. coli mastitis (35), but E. coli tend to persist in udders of cows with livers containing more than 28.3% fat. Vitamin E supplementation reduces the incidence of E. coli mastitis and selenium supplementation its duration (36); and a delayed neutrophil influx into milk and less efficient intracellular killing of E. coli was found in experimental mastitis in selenium deficient cows (37).

ELIMINATIVE BEHAVIOUR (DEFAECATION, DUNGING)

“Cattle deposit their excreta haphazardly with respect to location” (38). They make little or no effort to avoid walking through or lying in soiled areas except in so far as a freshly wet area may be cold and therefore uncomfortable. There is little to indicate that cattle are voluntarily in control of the passage of waste materials. Although defaecation may occur whilst the animal is walking or lying down, it most commonly happens when the animal is standing.

Animals whose young are born not fully formed (usually carnivores or omnivores) tend to live in “homes”, and avoid contaminating them with excreta. This does not apply to animals which evolved on grassland, and whose offspring are able to walk within hours of birth.

One of the great quantum leaps in the control of human disease was separation of excreta from drinking water and food. A number of disease problems in cattle are related to exposure to dung - a variety of foot conditions in housed cattle, environmental mastitis, worms and salmonellosis. Caged laying birds do not suffer from coccidiosis and parasitic worms in the same way as those on free range. In some racehorse studs, faeces are collected every day from paddocks as still the most effective method of controlling worms. It would be a major breakthrough if some efficient system of separating cows from their excreta could be devised. Presumably it would involve training, and there is the danger of welfare problems. A European method used for stalled cattle consists of an electrified wire across the top of cow stalls. A cow standing up and arching her back prior to defaecation and urination receives an electric shock; she will in future stand back in the channel prior to defaecation/urination. There was evidence of cleaner cattle, and better foot conditions, but also an increased incidence of anoestrus and other problems. The system has been banned on welfare grounds in some countries. However, if one can clearly present farmers with a defined problem they may devise an acceptable solution.

BEDDING

Bramley and Neave (39) correlated sawdust bedding “coliform” levels with udder new infection rate and suggested that mastitis problems arose when bedding “coliform” counts reached or exceeded 10^6/gm. Klebsiella pneumoniae caused 6/7 of the mastitis cases and predominated in the bedding. Bedding “coliform” numbers were static at 22°C, increased between 30-44°C and fell rapidly at 50°C. In more extensive studies a strong correlation was found between sawdust bedding K. pneumonia counts and K. pneumonia mastitis, but no
similar correlation for *E. coli* in the same herds (40,41). When sawdust bedding *E. coli* levels were maintained at 10^9/gm for 4 weeks there was an increased teat end contamination but no new “coliform” udder infections (42).

Milk leaking onto bedding from teats of high yielding cows may provide an excellent substrate for *E. coli* proliferation (43).

High yielding cows lie for longer periods than low yielders, especially in cold weather, and their body heat stimulates multiplication of *E. coli* in bedding (32). Visual assessment of bedding is a very inaccurate measure of bacterial numbers present.

Peri-parturient mastitis was associated with wet bedding in 90 Midlands herds (33). The correlation between dry bedding and absence of mastitis was high. High humidity, due to poor ventilation, overcrowding or weather increases bedding water content (44). A strong correlation between rainfall and sawdust bedding bacterial numbers was found for *K. pneumoniae* but not for *E. coli* (41). High humidity can be a major problem: optimal natural ventilation in cow housing is not sufficient to solve the problem in many parts of the country. Faecal *E. coli* counts of 10^8 have been recorded (23) and Faull et al. (34) described *E. coli* mastitis problems in a zero grazed herd receiving no bedding.

*E. coli* mastitis used to be considered a disease of housed cattle (45, 46), but in recent years a substantial incidence occurs during the summer. This could reflect changes in calving patterns (most dairy cows used to calf in October, and acute *E. coli* mastitis is a disease of early lactation), increased supplementary feeding during the summer or year round housing of high yielding cows.

**VACCINATION**

Historically, vaccination against mastitis has not been successful. Protective antibodies that appear in serum following vaccination do not appear in normal milk. They do enter the udder when it becomes inflamed, (when blood components enter milk). Thus they do not protect against intramammary infection, and a degree of mastitis (raised milk cell count) occurs before protection.

There are a very large number of serotypes of *E. coli*, as classified by surface antigens, and production of individual vaccines for every one would not be practical. However, the inner layer of the cell wall is common to the various serotypes of *E. coli*, (and all the Enterobacteriaceae). Naturally occurring outer cell wall deficient (rough mutant, “R”) coliforms are now used for vaccine production. These are the *E. coli* JF5 vaccine (47) and the Re-17 mutant *Salmonella typhimurium* bacterin toxoid (48). They appear not to prevent intramammary infection, but vaccinated herds have a decreased incidence of clinical mastitis due to “coliform” infections. It would appear therefore that in a herd with high *E. coli* challenge, even if vaccinated, cell count problems would remain. There would be a strong case for use of vaccines in herds with a clinical coliform mastitis problem. In one trial (49) a total of 67% of the Gram negative bacterial infections present at calving in control cows became clinical during the first 90 days of lactation compared to 20% in vaccinated cows. The vaccines appear to be safe, and do not have side effects such as suppressing milk yield.
REFERENCES