THE UDDER AS A POSSIBLE SOURCE OF COLIFORM ORGANISMS IN MILK

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The coliform group of bacteria has received considerable attention in milk sanitation, and a test for the presence of these organisms is included as a method for the official examination of milk samples in this country (*Min. of Health Memo.* 139/Foods, 1937). The sanitary significance of the presence of such organisms in milk has been questioned, however, by various workers in this country and in the United States (Ayers & Clemmer, 1918; Sherman & Wing, 1933; Chalmers, 1934; Wilson 1935; Yale, 1937; Stuart *et al.* 1938). These workers have objected to the test owing to its failure to indicate the difference between manurial and other sources of pollution. It is further emphasized that organisms of the group may proliferate rapidly in milk at temperatures above 50° F., and unless samples of fresh milk or of milk which has been stored at low temperature are examined, unsatisfactory tests may be the result of such proliferation rather than of initial infection.

Mattick & Williams (1925) and Malcolm (1933) have shown, however, that milk produced and handled under clean conditions contains few coliform organisms, and their findings have been supported by numerous other workers in this country. Mattick (1930) has stated that "The presence of coliform organisms in numbers in fresh milk is, therefore, evidence of carelessness at some stage in the handling."

Barkworth *et al.* (1927), Barkworth *et al.* (1929), Barkworth (1934) and Hoy & Newland (1931) have brought evidence to show that coliform organisms have an unfavourable influence on the keeping quality of milk, and other workers (Whitehead, 1930; Whitehead & Cox, 1932; Sherman & Wing, 1933; Pont, 1935; and Yale, 1937) have shown that the presence of these organisms in liquid milk and milk used for the manufacture of butter and cheese is undesirable.

Coliform organisms associated with mastitis

The possibility of infection of milk by coliform organisms present within the udder has received very little attention, and this is probably accounted for by the lack of evidence that such organisms are normal inhabitants of the healthy udder. There is evidence, however, that such organisms are sometimes associated with and presumably responsible for mastitis in dairy cows.

Munch-Petersen (1935) has tabulated the organisms isolated from cases of mastitis by different workers during the period 1890–1935 as follows:

Table I. *Distribution of organisms in cases of mastitis*

	No. of cases	%
Streptococci	13,197	80·64
Bact. coli	305	1·86
Bact. aerogenes	21	0·13
Mixed infections containing coliform organisms	66	0·40
Infections due to other organisms	2,777	16·97
Total	16,366	100·00

It is evident that coliform organisms are associated with only a small proportion of cases of mastitis in dairy cattle.

Savage (1907–8) found one acute case of mastitis apparently caused by coliform organisms out of a total of thirty-one.

Gilruth & MacDonald (1911) described the occurrence of mastitis in about thirty out of a total of forty cows in one herd. The symptoms were similar in all cases and from two infected udders *Bact. aerogenes* was isolated in pure culture. The disease was characterized by sudden onset with marked swelling of the infected quarters, the secretion being curdy and whey-like in appearance.

Jones (1918*a*, *b*) studied three cases of mastitis associated with coliform organisms, from two of which *Bact. coli* was isolated and from the third *Bact. aerogenes*. In two of the cases the milk contained numerous white floccules and in the third it had the appearance of haemolysed blood. One of the cases caused by *Bact. coli* recovered completely within 5 days, but in the other case the condition became chronic and the animal was slaughtered.

Lamont (1925) investigated twenty cases of mastitis and isolated coliform organisms in pure culture from one which developed rapidly in one quarter. The milk was a golden yellow fluid containing numerous white floccules. Coliform organisms were encountered in six other cases in mixed culture together with other organisms usually associated with mastitis.

Hardenbergh & Schlotthauer (1927) described seven cases of mastitis in one herd, and isolated *Bact. coli* from six cases and *Bact. aerogenes* from the other. The secretion at first was a clear fluid containing curd but was transformed within a few days into thick pus. All the cases occurred in one barn during a period of 6 months, but the connexion between the individual cases was not ascertained.

Rudolf (1928) found coliform organisms associated with twenty-four cases of acute mastitis out of a total of 535 examined. In one herd the acute inflammation disappeared in 1–3 days, and the milk resumed its normal appearance.

Minett *et al.* (1929) investigated 113 cases of mastitis, and isolated, from three, organisms of the coliform group. The secretion was markedly changed in appearance, excessively alkaline in two cases and normal in reaction in the

third. Organisms of the *Bact. coli* type were isolated from two cases, but from the third the organisms were regarded as belonging to the *Proteus* group.

Lesbouyries *et al.* (1933) found coliform organisms associated with thirty cases of mastitis out of a total of 200 examined. The milk of the affected animals had the appearance of clear, or sometimes yellow, whey with curds in suspension. The organisms isolated from four cases were closely related to, but not identical with, *Bact. coli tropicalis* (Castellani). It is interesting to note that the organisms were highly heat resistant, resisting 70° C. for 30 min. and 80° C. for 5 min.

Smith & Henderson (1934) isolated *Bact. coli* from one case of mastitis. This case developed suddenly and the secretion was markedly changed in composition, showing a high chloride and leucocyte content, and a decrease in the lactose.

According to Savickis (1936) coliform organisms are frequently associated with mastitis in Lithuania, and their presence in the udder secretion was demonstrated in 53·2% of a total of 325 cases investigated during the 3 year period 1933–5. During the same period the incidence in Latvia was observed to be 53·4% of a total of 388 cases.

Infection appeared in cows of all ages, and in all stages of the lactation, although cases were more frequent during the early stages. The milk in the majority of cases was a watery or yellow fluid containing curds; but in some cases it remained normal during the first 2 days following the appearance of clinical symptoms.

A total of 114 strains isolated from 110 cases were examined in detail. The majority of these were of the *Bact. coli* type, which was associated with a more acute clinical condition than was the case with the less frequent *Bact. aerogenes* infections. Symptoms resembling natural infection were produced when pure cultures in broth isolated from the infected quarters were injected up the teat canal. Similar symptoms, but of a less acute and of a more temporary character resulted from the injection of cultures derived from other sources.

Rowlands (1937) described a case of acute mastitis and isolated *Bact. aerogenes* in pure culture from the milk. This case developed suddenly, and the milk was a yellow whey-like fluid, containing curds. A fortnight later the milk was normal in appearance and coliform organisms were absent from 50 c.c.

Gwatkin *et al.* (1938) found coliform organisms associated with ten cases of mastitis, out of a total of 286. Four cases occurred within a few days of calving, the remaining six developing later in the lactation. In all cases the onset was sudden, and the milk from the six cases was markedly changed in appearance, in three being described as a yellow serous fluid containing curds.

Coliform organisms were isolated from ten other milk samples taken for mastitis examination, but in nine cases their presence was regarded as sampling contamination. In the other case *Bact. coli communior* was isolated from one quarter of a chronic mastitis case on two occasions 3 weeks apart.

The examination of samples taken a few hours after calving resulted in the finding of coliform organisms from one quarter in two cases out of a total of seventeen cows examined. No clinical disturbance of the udder was apparent, however, and the organisms disappeared from the secretion within a few weeks.

Yale (1937) states that: "Soon after an acute colon infection takes place, the milk becomes so clearly abnormal in appearance that it should be easily detected and kept out of the supply. Shortly prior to this, the milk may contain millions of colon organisms per c.c."

CONTAMINATION OF MILK WITH COLIFORM ORGANISMS FOLLOWING INJURY TO THE UDDER

Rowlands (1932) traced the source of coliform organisms in the bulk milk of one herd to a cow with an injured teat. Although preliminary examination of samples indicated that the udder itself was infected, it was shown that if the wound at the end of the teat was thoroughly cleaned and sterilized with methylated spirit it was possible to obtain from the quarter, milk free from coliform organisms in 1 c.c. samples. Bulk samples from this farm (thirty cows) had given repeatedly a positive presumptive test in three tubes of 0·01 c.c. and the farmer had nearly lost his Grade "A" licence as a result. The organisms isolated from the quarter samples were found to be identical with those isolated from the bulk milk. They were short Gram-negative rods, fermenting glucose lactose, saccharose and dulcite with the production of acid and gas, indole+, M.R.+ and V.P.−.

Egdell (1938) traced the presence of coliform organisms in "Tuberculin Tested" milk to one cow, that had damaged the right side of the udder on barbed wire. He writes:

"The right side quarters and teats looked quite healthy although they were scarred, and the whole slightly misshapen as a result of the injury. The fore-milk did not at first sight appear to contain any clots or flaky material; actually milk leaked slowly from the hind teat. After applying slight pressure over the scars on that teat and the corresponding quarter, the milk was found to contain traces of pus. Further pressure caused the scar on the teat to rupture outwardly with the escape of pus. A sample of the affected milk direct into a sample bottle gave a positive presumptive test in 0·0001 ml. (higher dilutions not tested) although the bacterial count did not exceed five figures.

"The milk from the fore-quarter was also contaminated with coliform organisms but not so severely. The organisms when typed gave typical *Bact. coli* reactions."

The cows were milked by machine, and the contamination of the bulk milk had occurred over a period of 6 months prior to its final detection.

Egdell has also encountered another case of milk from one quarter being infected with coliform organisms as a result of an injury to the quarter. At the time the case was detected, the only evidence of injury was a healthy scar at the juncture of the teat and the udder.

Druce (1938) has also observed a case of infection of milk with coliform organisms from a damaged teat.

Coliform organisms in milk from apparently normal udders

Chalmers (1934) studied the source of coliform organisms in the milk from a "Certified" farm. Samples of milk from this farm showed counts ranging from 250 to 1550 per c.c. with coliform organisms present in 0·1 c.c. during a period of 3 months. The organisms isolated from the milk samples were found to be heavily capsulated forms of *Bact. aerogenes*.

Chalmers states that the organisms were "gaining entrance to the milk in two ways, namely, from the outside of the udder, and also from an intermittent infection through the teat canal.

"Due to the capsulated nature of the organisms, ordinary methods of washing did not appear to remove them from the udder, and consequently a mild disinfectant was found necessary. The rejection of the normal amount of fore-milk did not appear to be sufficient to overcome the contamination of the milk."

It was observed, that on another farm producing milk free from coliform organisms, it was possible to draw milk free from such organisms in 10 c.c. from the udders of cows washed by ordinary methods.

Ritchie (1931*a*, *b*) traced the source of coliform organisms in the bulk milk from a "Certified" herd to the udders of two cows. Details of this case have been obtained from Cunningham (1938) who examined the milk samples. He writes:

"From about the end of October (1929) onwards the milk samples contained coliform organisms in 0·1 c.c. (three tubes) or less and showed a plate count on standard agar which was generally under 6000 and frequently under 3000 per c.c. About the end of February (1930), two cows (nos. 32 and 71) were found to be giving milk, which regularly contained coliform bacteria in 0·1 c.c."

The examination of quarter samples revealed that one quarter was infected in each case, the left fore in cow no. 32 and the right hind in cow no. 71.

Table II. *Samples from infected quarters*

		Coliform organisms (c.c.)			
Cow no.	Plate count	1	0·1	0·01	0·001
32 (L.F.)	196,000		+	+	+
		+	+		
			+	+	+
71 (R.H.)	1210		+	+	−
		+	+		
			+	+	−

"When the milk of these two cows was eliminated, samples of the bottled milk ceased to show the presence of coliform organisms in 0·1 c.c."

The organisms isolated from each case were short Gram-negative, motile rods, fermenting dextrose, laevulose and lactose with the production of acid and gas, but failing to ferment saccharose. Colonies on eosin-methylene blue agar were of the *Bact. coli* type. Further biochemical reactions of the organisms isolated are given in Table III.

Table III

No.	Indole	M.R.	V.P.	Citrate	Growth at 46° C.	Gelatine
32 (L.F.)	−	+	−	+	−	−
71 (R.H.)	+	+	−	−	+	−

Cow no. 32 was kept under observation until she calved and her milk in the next lactation was still found to be infected. "The coliform organisms isolated from the affected quarter were similar to those isolated in the previous lactation."

Ritchie (private communication) stressed the fact that the "udders of both cows were entirely normal throughout the time when infection was known to be present...it was quite impossible to find any difference on clinical examination between the infected quarter and the other three".

Westwater & Henderson (1938) traced the presence of coliform organisms in the bulk milk from a herd of thirteen cows to the udder of one cow. In a "clean-milk competition" the first four samples taken from this farm proved extremely satisfactory but four samples taken subsequently showed the presence of coliform organisms—two samples in 0·1 and two in 0·01 c.c.—although the bacterial counts still remained low. Further details of this case are not available but Westwater writes: "We demonstrated to our own satisfaction that coliform organisms were present within the udder of one of the cows."

The presence of coliform organisms in the bulk milk from a herd was recently investigated by the writer, and traced to infection within an apparently healthy udder.

The farm in question was of an exceptionally good type, milking about thirty cows and employing family labour of a very high standard of efficiency. The milk was sold to a dairy paying a bonus on the results of bacterial tests, and the results of tests during the period, October 1937 to April 1938 are summarized in Table IV.

Table IV

No. of samples	Bacteria per c.c.			No. of samples showing coliform organisms in (c.c.)			
	Max.	Min.	Mean	1	0·1	0·01	0·001
14	1460	270	924	9	4	1	0

On 13 April 1938, samples were drawn direct from the udder of each cow in the herd by Dunlop,[1] one of which showed the presence of coliform organisms

[1] This case was first detected by G. Dunlop, County Agric. Institute, Derby.

in 0·1 c.c. Quarter samples were taken from the suspected cow on four occasions. Sampling was carried out at three stages in the milking:

(*a*) After discarding first few streams of milk.

(*b*) Approximately half-way through milking.

(*c*) Strippings.

The presumptive *coli* test was positive in 0·01 c.c. on three occasions and in 0·001 c.c. on the other occasion in all the samples taken from the off hind-quarter. All the samples taken from the other three quarters gave a negative result in 10 c.c.

Samples of the milk were examined by various methods to ascertain if there was any evidence of disease. The results are given in Table V.

Table V

	Off hind-quarter	Bulk, other quarters
Chlorides %	0·10	0·095
Brom-cresol-purple	Normal	Normal
Acidity (% lactic acid)	0·165	0·165
Centrifugal deposit	Normal	Normal
No. of cells per c.c.	750,000	100,000
Catalase (10 c.c. of milk used)	0·75 c.c.	0·50 c.c.

The only evidence of abnormality is the higher cell count and catalase figure in the milk from the off hind-quarter as compared with the other three.

It should be noted that the cow aborted in July 1937, and at no time had given very much milk. At the time of sampling, the actual yield from the infected quarter was 2 lb., and it is estimated that if this quantity was included in a 12 gal. churn, the bulk would give a positive test in 1 or 0·1 c.c. The milk was, however, perfectly normal in appearance and there was no clinical evidence of disease in the infected quarter.

Since the removal of this cow from the herd, no difficulty has been experienced in producing milk free from coliform organisms in 1 c.c.

Cultures of the organisms isolated from the milk were: Indole+, M.R.+, V.P.−, Citrate−, Bile Salt Lactose at 44° C.+, Gelatine−.

Druce (1938) has also observed the presence of coliform organisms within the udder of a cow. The milk from the infected quarter was normal in appearance, but was positive by the brom-thymol-blue test. Mastitis streptococci were absent (blood-agar plate method) but coliform organisms were present in 0·001 c.c. The presence of this cow in the herd resulted in infection of the bulk milk, which disappeared when the animal was eliminated.

Morris (1938) traced the source of contamination of bulk raw milk to single cows with one or more quarters infected with coliform organisms. Six cases were encountered in different herds producing graded milk during the period 1930–7, *Bact. coli* being isolated from five and *Bact. aerogenes* from one. All the cases occurred during the late summer and autumn months. Following the removal of the animal with the infected quarter no difficulty was experienced in producing milk free from coliform organisms.

Samples from all six cases had a very high leucocyte content, were alkaline to brom-cresol-purple, and in two cases the chloride content was high. "There were no marked changes in the milk and it would have gone undetected by the ordinary milker."

Thomas (1938) isolated coliform organisms from samples taken aseptically from one cow. "Calves fed on this cow's milk developed scouring and three died." The udder was found to be clinically normal by the veterinary surgeon who took the samples, and the milk was perfectly normal in appearance. Cultures isolated from eosin-methylene blue agar plates were Indole −, V.P. +, M.R. +, Citrate +.

SUMMARY

1. It seems to be the general opinion of veterinary contributors on the subject that infection of the udder with coliform organisms is rapidly followed by clinical symptoms of mastitis, and marked changes in the appearance of the secretion. There would therefore appear to be little danger of contamination of bulk milk from such sources except during the few hours between infection and the appearance of the clinical symptoms.

2. Injury to the teats or quarters may result in infection of milk with coliform organisms owing to the difficulty of thorough cleaning of the malformed tissue.

3. Several cases of infection of quarters in the entire absence of clinical or other symptoms of disease have been cited. There is no evidence that such cases can be classed as subclinical forms of the disease as is the case in streptococcic mastitis. The evidence in two cases cited indicates that milk drawn from a quarter can remain infected with coliform organisms for a considerable period with no apparent clinical or other symptoms of disease.

4. There is sufficient evidence to warrant the examination of quarter samples in cases where infection of bulk milk with coliform organisms from other more common sources appears to be unlikely.

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