

EXCRETION OF *STREPTOCOCCUS PYOGENES* IN THE MILK OF NATURALLY INFECTED COWS

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(With Plate XII)

IN a recent article concerning the growth of *Streptococcus pyogenes* in milk at atmospheric temperatures, Pullinger & Kemp (1937) have set out the evidence which indicates that in milk-borne *Streptococcus* epidemics the immediate source of infection is the udder of the cow. These authors, in surveying the literature, cite nine of the instances—apart from those with which this paper is concerned—in which the cow or cows responsible for epidemics have been located; of these eight occurred in the United States and one in Great Britain. We take this opportunity of referring to another such case, which occurred in Sweden, an account of which is given by Rambe & Hedström (1934).

The epidemic started in February 1933, and of approximately 100 persons involved twenty-four showed a definite scarlatinal rash. The affected families obtained their milk from one farm, where a cow suffering from mastitis was found to be excreting beta-haemolytic streptococci, identical in their characters with those isolated from the patients. Important reactions of the organisms were: failure to split sodium hippurate, non-fermentation of sorbitol, pathogenicity for mice and rabbits. The cow was probably infected from the owner of the farm, who a week previously had suffered from a severely septic sore throat. At this time the cow had extensive sores on the hind-teats, and it was the hind-quarters in which the mastitis infection later developed.

Instances such as that just mentioned and those cited by Pullinger & Kemp are comparatively rare, either because the attempt to find the cow has been unsuccessful or, more frequently, because no attempt at finding her has been made. It is therefore not superfluous to give an account of two cows, which were known to have been responsible for milk-borne epidemics and which were afterwards purchased and kept under close observation. Although descriptions of the medical aspects of these two outbreaks have now been published, it is necessary to repeat some of the salient features and, by reference to these and to supplementary information which has been brought to our notice, we hope to throw further light on the circumstances under which the cows became infected and in turn contaminated the milk.

MILK-BORNE EPIDEMIC AT MØRKØV (1936)

An account of this outbreak has been published by Henningsen & Ernst (1938) and the facts regarding the herd (N.M.) which contained the infected cow (M36) are given below in chronological order.

20 September 1936. A clinical examination of the cows was made by Mr K. L. Jørgensen, and milk samples from the individual quarters of the seventeen cows comprising N.M.'s herd were taken for cultural investigation. Four cows were found to be infected in one or more quarters with *Str. agalactiae*. Another cow, M36, was excreting from the L.F. quarter numerous beta-haemolytic streptococci, which were identical in characters with *Str. pyogenes* of human origin and which were afterwards found to be similar serologically to the streptococci isolated from the two farm attendants and from the patients.

At this time no striking clinical symptoms of mastitis were observed in cow M36, though when her milk was allowed to stand in the laboratory that from the L.F. quarter was bluish and more transparent than the milk from the other quarters and the cream layer was mucous in character. Moreover, the L.F. milk was abnormally alkaline to brom thymol blue, showed a high catalase figure and a deposit of 2 parts per 1000 in the Trommsdorff tube.

October 1936, to January 1937. Cow M36, of the red Danish breed, was isolated on the farm until purchased by the College on 9 October. The L.F. quarter was at this time slightly smaller than the R.F. (Fig. 1) and somewhat indurated, especially near the base of the teat. The skin around the opening of the L.F. teat was reddened and showed several small fissures, while the left supramammary lymphatic gland was distinctly enlarged.

By the middle of November the L.F. quarter was practically dry, whereas the other quarters continued to secrete until the beginning of January. During this time also, the L.F. secretion gradually became more abnormal in character.

February to October 1937. The cow gave birth normally on 22 February to her third calf. Immediately after calving the L.F. quarter appeared to be of normal volume, but during the next few days it became moderately tender and swollen and slightly firmer than the R.F. The colostrum from the L.F. quarter was of a somewhat deeper yellowish colour than that from the other quarters; the milk secreted by this quarter was at first slightly flocculent; later, as it diminished in amount, it became more and more altered, until by July only about 100 c.c. of yellowish serous fluid containing clots could be obtained. During this period, too, there was progressive reduction in the size of the quarter. In Table I are given a few figures to illustrate the milk yield from the different quarters.

Microscopical examination of centrifuge deposit from the L.F. quarter nearly always revealed small numbers of streptococci, usually arranged as diplococci within leucocytes or as very short chains, while the deposit always contained very numerous polynuclear leucocytes. For the routine cultural examination, infusion agar containing 5% goat blood was used, but the haemolytic properties of the *Streptococcus* were equally well displayed when horse-blood agar was used. The numbers of streptococci estimated to be present in the fore milk on various dates are shown in Table II, the organisms apparently being in pure culture on almost every occasion. The streptococci were confined

to the L.F. quarter, the other quarters harbouring in the main harmless diphtheroids.

Thus, haemolytic streptococci were found at every examination from September 1936, to October 1937. On each occasion colonies were picked and grown in various test media with results as follows:

Medium Reaction	Blood agar beta-haemolysis 1% glucose broth (final pH) ca. 5.2	Litmus milk		Methylene-blue milk 0	Hippurate 0
		(37° C.) Slight acid	(10° C.) 0		
Medium Reaction		Lactose +	Sucrose +	Salicin +	Mannitol 0
Medium Reaction	Inulin 0	Raffinose 0	Trehalose +	Sorbitol 0	Aesculin 0

The reactions are clearly those of *Str. pyogenes* of human origin. A number of the cultures were tested on mice by intraperitoneal injection of 0.1 c.c. 24 hr. broth culture, and at this dose were invariably lethal, with streptococci detectable in the blood. Finally, through the kindness of Dr J. Ernst (Copen-

Table I. *Milk yield (in kilograms) of individual quarters of cow M 36*

	Left fore	Right fore	Left hind	Right hind
1936 October	0.39 0.22	2.81 3.44	3.51 3.21	3.5 3.16
November	0.15 0.09	2.61 2.76	2.76 2.76	2.71 2.76
December	0.04 0.07	2.49 2.29	2.36 2.16	2.44 2.24
1937 March	1.76 0.52	3.12 3.54	4.54 4.76	4.66 5.24
May	... 0.37	... 3.33	... 5.56	... 5.29
June	... 0.22	... 3.24	... 4.71	... 4.44
July	0.11 ...	3.24 ...	5.08 ...	4.01 ...

The milk was weighed weekly. The figures opposite each month show the average yield during the first and second halves of the month respectively.

... = no records available.

Table II. *Beta-haemolytic streptococci in milk of left fore quarter of cow M 36*

Date (1936)	9. x.	13. x.	16. xi.	20. xii.	22. xii.	30. xii.		
Numbers per c.c.	1000	3000	3000	150	3000	210		
Date (1937)	4. ii.	22. ii.	27. ii.	9. iii.	19. iii.	24. iii.	26. iii.	2. iv.
Numbers per c.c.	310	580	1000 to 20,000 (estimate)					
Date (1937)	22. iv.	22. v.	28. vi.	17. viii.	7. ix.	12. x.	26. x.	
Numbers per c.c.	60,000 (estimate)	1,000 to 20,000 (estimate)			12,760	120,000	820,000	

hagen), cultures isolated from the milk on 13. x. 36, 30. xii. 36, 4. ii. 37, and 22. ii. 37 were forwarded to Dr F. Griffith (London), who made a serological examination and reported that "there is no doubt that the four cultures are the

same and that they belong to group A (Lancefield). But I can find no evidence that they belong to any of the serological types which have so far been defined. In such cases one is always in doubt whether one is dealing with a non-specific variation or with a new type."

Further cultures isolated from the milk on 2. iv. 37, 29. vi. 37, 23. vii. 37 and 7. ix. 37 were examined by Dr Griffith, who reported that they gave some agglutination with his type XI and type XXVIII sera, though not sufficient to justify inclusion of the strains in either of these types. The difference in agglutination between these four strains and those examined earlier was not held to imply any difference in the strains as regards their type-specificity.

Cow M36 was slaughtered on 27. x. 37. She was in good bodily condition and four months pregnant. The L.H., R.F. and R.H. quarters appeared to be normal on naked eye and histological examination. The L.F. quarter was much reduced in size and its texture was tougher than normal. The mucous membrane of the sinus and larger milk ducts was thickened and its surface roughened. The left supramammary lymph gland was as large as the palm of a man's hand and about three times the size of the opposite gland. Scrapings from the cut surface of the L.F. quarter and from the larger milk ducts showed on cultural examination beta-haemolytic streptococci, the reactions of which were again verified as those of *Str. pyogenes* (of human origin). Streptococci could not be isolated from the supramammary, submaxillary or pharyngeal glands, while streptococci cultivated from the tonsils were different from those in the udder. Sections of the quarter showed a chronic productive inflammation; the interlobular connective tissue was greatly thickened and infiltrated with cells, while tissue with similar characters had invaded the lobules and destroyed many of the smaller ducts. In some of the ducts which remained, streptococci, mostly in the form of diplococci or short chains, could be easily found microscopically, mingled with polymorphs and monocytes.

Experiments on cattle

The pathogenicity for cows of the *Streptococcus* isolated from the L.F. quarter of M36 was tested in the following experiments:

21. xii. 36. The orifice of the R.H. teat of cow M36 was gently incised with a teat-slitting instrument, and on the two following days the tips of all the teats were moistened with secretion from the L.F. quarter. No infection was set up.

31. xii. 36. A glass rod moistened with broth culture of streptococci from the L.F. quarter of M36 was inserted into the teat canal of the R.H. quarter of cow W, an animal which was previously free from infection. Twenty-four hours later, cow W became feverish and at the same time an acute mastitis developed in the R.H. quarter. In the course of a few days the general symptoms subsided, but the R.H. quarter was ultimately destroyed and the right supramammary gland became much enlarged. Group A streptococci were repeatedly isolated from the R.H. quarter, while on 19. iii. 37 mastitis developed in the R.F. quarter of cow W, and this was found to be due to streptococci of the same type as those introduced into the R.H. quarter.

Cow W calved on 5. iv. 37, and the calf was allowed to suck the mother until it was killed

on 11. iv. 37. The calf was then apparently normal and well-nourished. Haemolytic streptococci identical with those found in the R.H. and R.F. quarters of cow W were isolated from the tonsils and from a submaxillary and retropharyngeal lymphatic gland, though the number of streptococci in the glands was apparently very small. Cow W was also killed on 11. iv. 37. Haemolytic streptococci were isolated from the two diseased quarters but not from other situations examined, viz. the superficial inguinal, pharyngeal and submaxillary glands, the tonsils, the uterine mucosa.

30. xii. 36. The orifice of the R.F. teat of cow 11, hitherto free from *Streptococcus* infection, was gently slit and next day all four teats were moistened with the same broth culture as was used for cow W. Infection failed to appear.

21. i. 37. Since no infection had appeared when the *Streptococcus* from the L.F. quarter of cow M36 was applied to the slightly injured teats of cow M36 R.H. and of cow 11 R.F., a similar experiment was performed, using a strain of *Str. agalactiae* freshly isolated from a case of mastitis. A slight injury was inflicted with the teat-slitter at the orifices of the R.F. teat of cow 11 and the L.F. teat of cow W. In the course of the next 24 hr. the skin of these teats was moistened with a 24 hr. broth culture of the *Streptococcus*. In both cases infection occurred, clinical signs of mastitis appearing in the inoculated quarters of cow 11 1 week later and of cow W 8 weeks later. This experiment served to show that cows 11 and W were not specially resistant to ordinary mastitis streptococci. It is also of interest to note that in cow W the L.F. quarter showed clinical signs of mastitis due to *Str. agalactiae* at the same time that mastitis due to *Str. pyogenes* appeared in the R.F. quarter.

MILK-BORNE EPIDEMIC AT DONCASTER (1936)

The main features, as reported by Watson (1937), were as follows. The epidemic, which lasted from 9 to 20 December 1936, was due to raw milk retailed from a single farm with a daily output of 50 gal. Of 380 families, comprising 1343 persons, 205 (54%) were affected in greater or less degree, and in these families there were 364 cases of tonsillitis or of scarlet fever. Two or possibly three deaths were attributed to the outbreak. Most of the cases occurred between 10 and 17 December, by far the largest number being reported on 10, 11 and 12 December, and there was a considerable drop in the number reported on 13 and 15 December. Other facts which are of significance for the present purpose are set out below in chronological order. The information is obtained from the report by Dr Watson, by personal communications from Dr F. Griffith (Ministry of Health) and Mr D. D. Canning, M.R.C.V.S. (Veterinary Inspector, West Riding County Council), and by our own observations.

12 December. Inquiry at the farm showed that one of the milkers had had a sore throat "for about a week". It was afterwards learnt that this milker had a child who had been suffering from otorrhoea since 2 December, and that the milker had been dressing the ear.

15 December. (1) Pasteurization of the milk supply of the herd became effective as from midday.

(2) Ear secretion from the milker's child and swabs from the milker's throat and from seven of the patients were examined by Dr Griffith, and from all scarlatinal streptococci belonging to Griffith's serological type II were isolated.

(3) An inspection of the thirty-two cows forming the herd was carried out by Mr Canning, who reported that none of the cows was showing clinically obvious mastitis. One cow, however, known as the "red cow", had an induration in the R.F. teat, though the quarter itself and the milk were normal. According to the owner, this teat had been crushed about a fortnight previously by a tread from another cow, and after this the secretion from the quarter was altered for a few days.

(4) Mixed samples of milk were taken from groups of four cows, and submitted to Dr Griffith, who isolated by mouse inoculation type II streptococci from the milk of the group containing the red cow.

19 December. (1) Mr Canning reported that the red cow was now showing signs of mastitis in the R.F. quarter, but that the quarter was not very painful. The other quarters, including the L.F., were showing no signs of mastitis.

(2) Samples of mixed milk from the four quarters of the red cow revealed type II streptococci by mouse inoculation.

23 December. Clinical examination (Mr Canning) and bacteriological examination (Dr Griffith) of the individual quarters and milk of the red cow gave the following results:

R.F. quarter: swollen and its secretion purulent. Type II streptococci were isolated on blood agar and by mouse inoculation.

L.F. quarter: *normal clinically*. Type II streptococci were isolated from the milk by mouse inoculation.

R.H. quarter: said to be showing a slight mastitis. A streptococcus isolated from the milk was not of type II.

L.H. quarter: normal clinically and bacteriologically.

The remaining portions of the milk samples were forwarded to the Institute and examined by Dr A. W. Stableforth. Centrifuge deposits were plated in ox-blood agar, and the secretion from the R.F. quarter was found to contain beta-haemolytic streptococci, estimated to number about 10 millions per c.c. These were identified as belonging to Lancefield's group A. No beta-haemolytic streptococci were grown from the L.F. milk or from either of the other quarters.

28 December. A bulk milk sample from the herd after removal of the red cow was examined by Dr Griffith with negative results.

29 December. (1) Mr Canning reported that both forequarters of the red cow had rapidly atrophied and that the induration in the R.F. teat was still quite marked.

(2) Mr Canning sent to the Research Institute milk samples from individual quarters of all cows in milk, thirty-one in number, including the red cow. Centrifuge deposits from these milks were plated in ox-blood agar, with the result that twelve quarters of five cows were showing haemolytic streptococci, including the two fore quarters of the red cow. The streptococci from the red cow were fatal to mice on intraperitoneal inoculation of 0.1 c.c. 24 hr. serum broth culture, and belonged to type II. None of the remaining cultures was

virulent for mice, and in other respects also, e.g. hippurate tests, final pH in 1 % glucose broth, they behaved like *Str. agalactiae*.

5 January 1937. The red cow arrived at the Research Institute. She was in good condition and was said to be 14 weeks pregnant with the third calf.

6 January. Both fore-quarters were found to be greatly atrophied, especially the R.F. (Fig. 2). The R.F. teat was firm and swollen and rather doughy to the feel. Clotted material could be obtained from both fore-quarters, but only with difficulty from the R.F.; the milk from the two hindquarters was normal in appearance. Cultural examination (ox-blood agar) showed, in the R.F. quarter, *Staphylococcus aureus*, in the L.F. quarter, type II streptococci in small numbers—estimated at 440 per c.c.

11 January. A small amount of clotted material obtained from the R.F. quarter yielded *Staph. aureus* only.

22 January. The R.F. quarter was now quite shrivelled. From the L.F. quarter about 280 c.c. of thick clotted material was obtained, and this on microscopical examination showed numerous Gram-positive diplococci. Cultural examination yielded type II streptococci in numbers estimated at 8000 per c.c.

28 January. About 140 c.c. thick material was got from the L.F. quarter, and this on microscopical examination showed numerous diplococci. Type II streptococci were isolated by cultivation in serum glucose broth. The streptococci killed mice inoculated intraperitoneally with 0.1 c.c. broth culture.

1 February. 270 c.c. creamy material obtained from the L.F. quarter; examined microscopically, small numbers of diplococci could be seen, but no growths were obtained in blood agar or in glucose broth.

4 February. 80 c.c. thick creamy material obtained from the L.F. quarter. Samples representing the first and last parts of this were examined separately. Small numbers of diplococci could be seen in the secretion, but no growths were obtained in ox-blood agar or in glucose broth.

6 February. 50 c.c. rather watery material got from the L.F. quarter. A sample representing the last part of this, examined microscopically, showed a few diplococci. Centrifuge deposit from this sample sown in serum glucose broth yielded a culture of streptococci, but unfortunately these could not be grown in subculture.

10 and 15 February. On each occasion about 50 c.c. rather watery milk was obtained from the L.F. quarter. Microscopically this showed a few diplococci, but the organisms could not be cultivated in blood agar or in serum glucose broth.

It is unnecessary to relate the further history in detail. The cow gave birth to a bull calf on 4 July 1937, and between then and the date of slaughter, 28 July 1937, milk from the L.F., L.H. and R.H. quarters was examined on nine occasions. Each time, 0.5 c.c. gravity cream, 1.0 c.c. whole milk, and the centrifuge deposit from 10.0 c.c. milk were plated in ox-blood agar. In addition, in the case of the L.F. milk, some cream was sown in serum glucose broth and

two mice were injected intraperitoneally, each with 0.5 c.c. whole milk. Growths appearing in the liquid medium were plated out in blood agar. From the R.F. quarter only a small quantity of clotted material could be obtained, and this also was cultured in ox-blood agar, in serum glucose broth, and injected into mice. The result of all these examinations was uniformly negative for haemolytic streptococci, and at this time the R.F. quarter was heavily infected with *Staph. aureus*.

On post-mortem examination, the cow was found to be tuberculous. The L.H. and R.H. quarters of the udder appeared to be normal on being sliced. The R.F. quarter, on the other hand, was greatly reduced in size and its substance very tough; the lining of the R.F. teat cistern was roughened owing to the formation of coarse scar tissue. Microscopically, the lining epithelium was denuded in places. The L.F. quarter also was "atrophied", and its substance denser than normal, but the lining of the L.F. teat cistern appeared to be normal. A final bacteriological examination of the L.F. and R.F. quarters was made by culturing scrapings from the cut surfaces and fluid pipetted from the teat sinuses and by injecting the fluid into mice, but no haemolytic streptococci could be demonstrated.

DISCUSSION

The outbreaks at Mørkøv and Doncaster presented no very unusual features, and we were fortunate in securing the cows responsible. Subsequently, one of our chief objects was to see how long the streptococci would persist in the udders of these cows and also whether they would survive the dry period. Several points of interest and importance emerge. Thus, there is the extremely long persistence of infection in the L.F. quarter of cow M36, viz. for at least 13 months, including a dry period of about 3 months, and longer than has been reported previously in such cases. Indeed, group A streptococci were still present in the gland at the time of slaughter. With the red cow (Doncaster) two quarters were involved, first the R.F. and a little later the L.F., but the duration of excretion did not exceed some 6 weeks, probably owing to the great intensity of the reaction. With both cows, however, the final histological changes in the udder were not very different.

Two other features illustrate the dangerous character of these cows. Both at times showed a high *Streptococcus* count in the milk, and both passed through stages when the clinical signs of infection were not obvious. With the red cow, the information given on the various dates indicates the important fact that in both quarters there was a pre-clinical stage lasting several days, although the milk was known to be infective. With M36 the symptoms were by no means striking and the case might well have passed for one of ordinary clinical mastitis due to *Str. agalactiae*. The owner, in fact, was not impressed with the seriousness of the position and was rather disinclined to sell the cow, which he regarded as one of his best animals.

With the red cow, there was clearly an association between injury to the R.F. teat and onset of mastitis in that quarter. With M36, at the time of purchase the teat of the affected quarter was seen to be fissured but it is not known whether this preceded the mastitis. One of us (Bendixen, 1934) has shown by experimental means the importance in the spread of mastitis of injuries about the teat orifices. It has also been pointed out (Minett, 1937) that such injuries may well be a decisive factor in connexion with the origin of milk-borne *Streptococcus* epidemics. It seems that for injuries to be effective in this sense they must be severe, e.g. such as may be caused by a tread; minor wounds about the teats are extremely common, but in herds associated with epidemics not more than one or two cows become infected. Milk-borne *Streptococcus* epidemics occur most commonly during the winter months, and in explanation of this there seem to be two contributing factors, first, that severe teat injuries are more liable to occur in the winter when cows are housed at night and, second, that throat affections among human beings have a higher incidence during the colder weather. Hence, in the prevention of epidemics, apart from pasteurization of milk supplies, several points are involved: (1) the exclusion as far as possible of persons suffering from throat ailments, (2) machine milking, where economically possible, (3) the provision of adequate floor space for cows, (4) prompt isolation and treatment of cows with injured teats. Attention to these four points may reduce but will not of course eliminate the risk.

The facts brought out in this paper illustrate once again the immediate responsibility of the cow in epidemics of this sort. The idea that these epidemics arise through direct contamination of milk by human beings is one which for various reasons is deeply rooted, in spite of evidence to the contrary. Even those who have been impressed by the importance of indirect transmission of infection by the cow have been tempted to compromise by admitting that the smaller outbreaks may result from direct contamination of the milk. Now that Pullinger & Kemp (1937) in an exhaustive work have shown that *Str. pyogenes* is incapable of growing in milk at atmospheric temperatures, it is extremely doubtful whether even this compromise should be admitted.

SUMMARY

An account is given of two cows which were concerned in milk-borne *Streptococcus* outbreaks at Mørkøv (Denmark) and Doncaster (England) respectively, and which were afterwards kept under close observation. The Danish cow excreted *Str. pyogenes* from the affected quarter for at least 13 months, including a dry period of about 3 months; the other animal excreted similar streptococci for about 6 weeks. In both cases there were stages when symptoms of mastitis were either not evident or not pronounced, although the milk was known to be infective.



Fig. 1. Cow M36 on 10. x. 36. Left fore quarter "atrophied".



Fig. 2. Doncaster cow. "Atrophy" of both fore quarters.

ACKNOWLEDGEMENTS. We gratefully acknowledge assistance received from the following: Dr Th. Madsen, Dr J. Ernst, Dr E. J. Henningsen and Mr K. L. Jørgensen (Copenhagen), Drs R. Watson and A. Penman (Doncaster), Mr D. D. Canning (West Riding County Council) and Dr A. W. Stableforth (London). Our special thanks are due to Dr F. Griffith (Ministry of Health, London) for providing data on the Doncaster outbreak and for making a serological examination of cultures from the cows.

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(*MS. received for publication 4. XII. 37.*—Ed.)