

Staphylococcal food poisoning from sheep milk cheese

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SUMMARY

Cheese made from sheep milk was implicated in food-poisoning incidents in December 1984 and January 1985. Bacteriological examination of batches of cheese failed to reveal a viable pathogen but enterotoxin A produced by *Staphylococcus aureus* was present. This was the first time that enterotoxin was detected in a food produced in the UK which was associated with poisoning and from which viable *Staph. aureus* could not be isolated. Subsequent detailed examination of milk, yoghurt and cheese from the same producer revealed that contamination with *Staph. aureus* was associated with post-infection carriage as well as clinical illness in ewes on the farm. Strains producing enterotoxin A were still intermittently present in the bulk milk used for cheese production nearly 2 years afterwards, apparently in the absence of clinical illness in the sheep. The possible effects of heat treatment are discussed. Any changes in legislation should cover all non-human mammalian milk used for human consumption.

THE OUTBREAK

On 31 December 1984 approximately 170 resident guests and 65 non-resident guests attended a Hogmanay dinner in a hotel in Ayrshire. Thirteen residents (three being hotel staff) reported illness, the onset of which varied between 3 and 6 h after the meal. The principal symptoms were of violent vomiting followed by severe diarrhoea. This lasted for 2–6 h and there was rapid recovery. During subsequent enquiries by the local Department of Environmental Health it was found that after a 'gourmet' meal on 5 December 1984 12–14 other persons had suffered similar symptoms. Only smoked scallops and sheep milk cheese were common to both meals, and 6 of the 13 ill on 31 December had not eaten the scallops. Approximately 60 portions of cheese had been served on this occasion, but not all of those present had eaten the sheep milk cheese which was not part of the normal hotel cheese board. Portions of a pasta course, a beef course, a cheese course and a sweet were submitted for analysis to Crosshouse Hospital, Kilmarnock. Environmental swabs from the kitchen were also examined. No pathogen was recovered.

The opinions expressed are those of the authors and do not necessarily represent those of their employing authorities.

On 8 January 1985 Knowsley Metropolitan Borough Council (Kirby, Liverpool) reported that a patient had suffered unspecified 'food poisoning symptoms' after eating sheep milk cheese made at the same establishment as that implicated in the previous outbreaks. The patient, a doctor, had submitted cheese to his local laboratory but no pathogen had been found.

Previously in August 1984 two persons in Dumfries had complained of vomiting and diarrhoea within 12 h of eating cheese made in March/April of that year, but investigations had been inconclusive and neither *Escherichia coli* nor *Staphylococcus aureus* was present. Laboratory investigations to this point had demonstrated very high total viable bacterial counts ($> 5 \times 10^8$ /g of cheese), mainly streptococci, including faecal streptococci, but also large numbers of coliforms (2.5×10^5 /g of cheese).

INVESTIGATION AT THE FARM DAIRY

The cheese was manufactured in the Annandale and Eskdale District of the Dumfries and Galloway Region. Environmental health officials from that District continued the investigation. The producer was visited and a voluntary agreement obtained to ensure that no cheese would leave the store after 8 January 1985, to allow further investigations to take place.

The proprietors of the farm had previously carried out cheese production in different premises, and had moved to these premises only within the previous 2 years. Existing farm buildings had been converted to form a 24-stall sheep milking parlour with refrigerated bulk milk tank, cheese-making rooms and a refrigerated store. Milk production was confined to the months of March to October, a flock of 71 Friesland sheep giving approximately 20–25 gallons of milk per day.

Cheese of batch 81/84 implicated in the Knowsley incident had been supplied only to outlets in Edinburgh and London, yet the patient had been given this as a gift obtained in Glasgow. Sub-retailing between delicatessens is apparently common. All of this batch had been distributed and could not be traced. The milk at this farm had been monitored by the local Environmental Health Department as part of their normal surveillance programme, and it was noted from their records that milk samples taken from the bulk milk tank on the day of production (19 June 1984) had contained large numbers of *E. coli* (2.6×10^5 /ml). Batch number 82/84 was taken as the nearest alternative sample for laboratory testing. The hotel in Ayrshire had been supplied indirectly by a delicatessen in Glasgow to which all of batches 149–155/84 were sent. None of these smaller cheeses remained in store. Batch 168/84 (16 September 1984) was sampled as the nearest alternative.

When the presence of staphylococcal enterotoxin A was reported by the Food Hygiene Laboratory, Central Public Health Laboratory, London (1), on 22 January 1985 the proprietor was served with a Notice under Section 24 of the Food and Drugs (Scotland) Act 1956 (2) prohibiting 'any sheep milk or product derived thereof being removed from the premises', as there were 'reasonable grounds to believe that these goods were likely to cause food poisoning'.

Further detailed sampling was then commenced to ascertain the magnitude of the contamination. Initially a single cheese was examined from each batch remaining in store.

CHEESE PRODUCTION

Precise records were not available for examination. The routine appeared to be as follows: the milk from the morning milking was mixed in the bulk tank with the milk of the previous evening which was held at 5 °C, and the mixture was then passed by pipeline to the adjoining but separate cheese-making room. Cheese production was begun by heating the milk to approximately 32 °C, which was thought to take 2 h, and then holding the milk at this temperature for approximately 1 h before the starter culture (propagated on the farm) was added to each batch. The curd was produced by the addition of a commercial rennet, drained and pressed in cloth (in stainless steel presses with wooden followers) for 48 h. The resulting cheese was then air dried for 4 or 5 days at about 16 °C before being wax-coated and stored at a maximum 16 °C for a minimum of 6 weeks before sale, mainly to delicatessen outlets. Two sizes of cheese were produced, 0.5 and 1.5 kg.

INVESTIGATION OF CHEESE IN STORE

The numbers of viable *Staph. aureus* and of *E. coli* 1 were determined in the cheese. Since examination for enterotoxin was expensive and time-consuming an indicator of staphylococcal growth was required, staphylococcal thermonuclease being chosen. A random sample of those cheese which contained thermonuclease together with some negative controls were sent to the Food Hygiene Laboratory, London to be tested for the presence of enterotoxins. As well as enterotoxin A, large numbers of *E. coli* 1 were intermittently present. Since a standard was required by which to judge these results various other cheeses were taken at random from local retail outlets. Using the same methods described elsewhere, counts were performed for total coliforms, *E. coli* 1, and *Staph. aureus*.

A meeting was convened on 1 May 1985 by the Director of Environmental Health with the proprietors and a large number of advisors, including the veterinary general practitioner serving the farm, the Veterinary Investigation Officer, the Divisional Veterinary Officer, the Community Medicine Specialist and the Consultant Bacteriologist to the Health Board, together with representatives from the West of Scotland Agricultural College, the Department of Agriculture and Fisheries, and the Communicable Disease (Scotland) Unit.

The Divisional Veterinary Officer noted that there had been staphylococcal mastitis on the farm since June 1984. The veterinary general practitioner had attended a small number of animals with clinical mastitis affecting one quarter and had advised that the ewes be withdrawn from milking whilst showing clinical illness. The farmer had diagnosed mastitis in a number of other ewes, but it appeared that milk drawn from the unaffected quarters continued to be added to the bulk milk used for cheesemaking. The diagnosis seemed to rest upon the nodular texture of the udder and a streaky appearance of the milk. No information was available about the total number of animals affected. The proprietor had been advised to institute bacteriological quality control, but the mastitis had continued, *Staph. aureus* having been isolated on 22 March 1985 (phage type 54; enterotoxin A-positive). It was agreed that the veterinarians would help the farmer to identify and isolate those sheep which could constitute a risk to cheese production,

including those with minimal signs of clinical illness. The cheese production was to be altered under the guidance of the West of Scotland Agricultural College. Screening of cheese samples produced under a new regimen would hopefully allow the order of prohibition to be lifted.

The main changes in cheese production were to be as follows: reliable and rapid refrigeration of newly produced milk, more rapid warming of the milk to fermentation temperature and addition of commercially produced starter culture. The producers were strongly against any form of heat treatment of the milk, feeling that this detracted from the commercial value of their product.

MATERIALS AND METHODS

Enumeration of total bacteria

Milk and yoghurt were tested by methods described in the Milk (Special Designations) (Scotland) Order 1980 (1980 no. 1866). The methods used for cheese were similar, but an initial emulsification was required; equal weights of cheese and quarter strength Ringer's solution (Oxoid no. BR52, Oxoid, Basingstoke, England) were emulsified using a Colworth Stomacher 400 machine (A. J. Seward, Bury St Edmunds, Suffolk, England), and a primary dilution of 1 in 10 obtained. Quarter strength Ringer's solution was used as the diluent for all subsequent culture work; tenfold dilutions of cheese were prepared and 1 ml of each dilution was plated in duplicate on Milk Agar (Oxoid no. CM21). Plates were incubated at 30 °C for 72 h. The dilution showing discrete colonies on culture was chosen for enumeration. All colonies were counted and the arithmetic mean count calculated.

Presence of coliforms

Using the same dilutions as used for the total bacteria count, 1 ml was dispensed into three tubes, each of which contained 5 ml MacConkey broth (Oxoid no. CM5a) and an inverted Durham's tube. After incubation for 72 h at 30 °C those specimens showing acid and gas production in two or more tubes were deemed positive. Subcultures from those positive tubes were tested for acid and gas production after incubation at 44 °C for 24 h, and designated 'typical' *E. coli* A representative sample of those were confirmed as *E. coli* 1 by Analytical Profile Index 20E (API Système SA, Vercieu, France).

Presence of E. coli 1

1 ml of an appropriate dilution was added to 4 ml of MacConkey Agar no. 3 (Oxoid No. CM115), and the mixture allowed to set on a roll tube machine (approximately 1–2 min). After incubation for 24 h in a water bath at 44 °C those colonies showing acid were deemed positive, and the count obtained from those tubes showing discrete colonies.

Enumeration of staphylococci

One millilitre of the appropriate dilution was plated on duplicate plates of Baird–Parker medium (Oxoid no. CM275) and Columbia horse blood agar (Oxoid no. CM331). After incubation for 48 h at 37 °C colonies showing typical appearances were counted and confirmed as *Staph. aureus* by reactions to

coagulase (slide and tube methods) and for the production of thermonuclease. Representative strains were submitted to the Central Public Health Laboratory, London to be examined for enterotoxin production and phage typing.

The thermonuclease test

Most strains of *Staph. aureus* produce a heat-stable DNA-ase, which tolerates heating at 100 °C for 15 min. The nuclease was detected by the slide procedure of Lachica (3).

pH testing

A 10^{-1} dilution of cheese in normal saline was prepared using the Colworth Stomacher 400 machine. pH was measured using a Corning EEL Model 109 pH meter with a Russel type CE74 electrode (Corning Limited, Stone, Staffordshire, England). During the initial investigations there was shown to be no significant difference between pH measured using quarter strength Ringer's solution as the diluent in place of saline, consequently tests were conducted only with Ringer's solution when large numbers were subsequently tested.

The differences in pH measurements from samples taken before and after the changes in the methods of cheese production (June 1985) were analysed using the student's *t* test.

RESULTS

Examination of samples 82/84 (date of production 19 June 1984) and 168/84 (date of production 16 September 1984) taken on 8 January 1985 showed pH of 6.1–6.2, and high total coliform counts (1.3×10^6 to 9.0×10^7). Viable *Staph. aureus* was not detected. However batch 82/84 contained *E. coli* 1 (5.5×10^3), was also thermonuclease positive, and was subsequently shown to be positive for enterotoxin A. As described earlier a selection of cheeses from local retail outlets was tested by the same methods. These included Cheddar, Edam, Brie, Gruyère, Austrian smoked, Danish Blue, and Camembert. *Staph. aureus* was not detected in any cheese, and only one sample (Camembert cheese) contained *E. coli* 1 (10^3 /g of cheese). This cheese also displayed a high total coliform count (4.1×10^6 /g).

A sample consisting of a single core from each batch of cheese produced in 1984 remaining in store on 14 February 1985 was examined for *E. coli* 1, *Staph. aureus* and thermonuclease. A representative selection of those which were thermonuclease positive was submitted to the Food Hygiene Laboratory, London for enterotoxin tests. *Staph. aureus* was detected in 2 of the 78 cheeses examined but 17 were positive for the presence of staphylococcal thermonuclease. Phage typing was not performed. Enterotoxin A was present in 4 of 9 batches which were negative for *Staph. aureus*. One of these was also negative for thermonuclease.

On three occasions yoghurt was tested on the day of production. On 18 March 1985 small numbers of *Staph. aureus* were detected (< 10 /ml). This culture produced enterotoxin A and was phage type 54, which was also later isolated from cheese 11/85 produced from the same batch of milk (Table 1).

Since *E. coli* 1 had not been shown to be a frequent contaminant of the milk, suspicion was directed towards the cheese starter culture which had been propagated by the cheesemaker without bacteriological control. When tested on

Table 1. To determine persistence of *Staph. aureus* in cheese after production serial counts on cheeses known to contain viable organisms (% of count at 1 week shown in parentheses)

Batch no.	Weeks after production (numbers of <i>Staph. aureus</i> /g)			
	1 week	2 weeks	4 weeks	6 weeks
11/85	75×10^6 *	4×10^6 (5.3)	110×10^3 (0.14)	100×10^3 (0.13)
48/85	60×10^3	60×10^3 (100)	1×10^3 (1.6)	0 (0)
49/85	140×10^3	140×10^3 (100)	18×10^3 (12.8)	500 (0.35)
50/85	8×10^3	8×10^3 (100)	2×10^3 (25)	2×10^3 (25)
51/85	12×10^3	12×10^3 (100)	1.6×10^3 (13.3)	0 (0)
52/85	80×10^3	40×10^3 (50)	10×10^3 (12.5)	1.2×10^3 (1.5)

* Enterotoxin A produced. Phage type 54. pH 6.34 (other batches not tested).

Table 2. Mature cheeses tested prior to release for retail 17 September 1985 (N.B. new commercial starter used)

Batch date	<i>Staph. aureus</i> count per g of cheese	Thermonuclease
8 June	< 50	—
16 June	< 50	+
21 June	< 50	+
30 June	< 50	+
14 July	< 50	—
23 July	< 50	—
27 July	7.5×10^4 *	+
7 Aug.	1.2×10^3 †	+
18 Aug.	< 50	—
21 Aug.	< 50	—
23 Aug.	< 50	—
25 Aug.	< 50	—
27 Aug.	< 50	—
31 Aug.	< 50	—

The average pH was 5.53 (range 5.31–5.61).

* Phage non-typable, enterotoxins C and TSST1 produced.

† Phage typing not done.

2 May 1985 large numbers of *E. coli* 1 were present (1.3×10^8 /ml). *Staph. aureus* and thermonuclease were not detected. The pH was 5.7.

Milk was examined on 26 occasions between 17 April and 29 October 1985. On 15 occasions *Staph. aureus* was present (median count 10/ml; range 0–136/ml). Three random isolates were tested for enterotoxin production; two produced enterotoxin A (17 April 1985, 23 April 1985), the other both enterotoxins C and TSST1 (30 April 1985). All were phage non-typable. Although milk sampled on 23 July 1985, 30 July 1985, and 6 August 1985 contained no detectable *Staph. aureus*, cheeses produced on 23 July 1985 and 7 August 1985 and tested on 17 September 1985 also contained *Staph. aureus* (Table 2). The strain from 23 July produced both enterotoxins C and TSST1. It too was phage non-typable.

Staph. aureus was isolated from three members of staff at the dairy, but the strains were shown to be different from those detected in the foods, one producing

both enterotoxins A and TSST1 (phage type 29/52), one TSST1 only (phage type 81), and one producing no enterotoxin (phage non-typable).

Cheese production had recommenced in 1985 with the spring milking, although no cheeses had been released. Between 13–18 May 1985 a core sample was examined from a single cheese from each of 47 batches in store (comprising 328 cheeses). All but one of these core samples contained *Staph. aureus* in large numbers (median count 40×10^3 /g; range $0\text{--}4 \times 10^6$ /g). The mean pH was 6.2 (range 5.88–6.86). *E. coli* 1 was present in all samples (range 1.6×10^3 to 4×10^6 /ml). On 26 June 1985 core samples were tested from 367 cheeses (i.e. all cheeses in store, and including repeat tests on batches previously tested). *Staph. aureus* was detected in only four, indicating that *Staph. aureus* numbers decline with maturity of cheese.

Since a new recommended regimen had been followed from the beginning of June 1985 14 mature cheeses were examined prior to release to retail outlets. A new commercial starter had been used. *E. coli* 1 was not detected in these samples. The results are shown in Table 2. The reduction in mean pH (from 6.2 to 5.53) was highly significant when compared to production prior to June 1985 ($P = < 0.0005$).

To determine the mode of decline of *Staph. aureus* from cheese during maturation serial counts were performed on cheeses known to contain viable organisms. The results are shown in Table 1. On this basis, at 6 weeks these cheeses would have satisfied the standards proposed by Collins-Thompson and colleagues (4).

Finally, a random sample of milk examined on 28 May 1986 contained a strain of *Staph. aureus* which produced enterotoxin A but this strain was phage non-typable.

DISCUSSION

Skin sepsis and mastitis due to *Staph. aureus* are well recognized in sheep, though enterotoxin production by such strains (most commonly type C) does not apparently cause clinical problems (5). Enterotoxin A is most commonly associated with food-poisoning in man (1), and staphylococcal food poisoning was thought to be the main cause of foodborne disease in Spain where most sheep milk is converted into cheese (6). A review of the literature shows that staphylococci were encountered less often in cheeses free of coliforms (7). Toxic cheeses contain upwards of 1.5×10^6 /g *Staph. aureus* (8), and enterotoxin was distributed uniformly throughout each vat of cheese which Zehren & Zehren examined (9).

Minor & Marth (10) studied the effects of gradually reducing pH on the growth and survival of staphylococci and confirmed that at pH 4.6 lactic acid was bactericidal for *Staph. aureus*. As this outbreak shows *Staph. aureus* is a significant cause of illness and isolation of the bacterium alone is an inadequate indicator. We demonstrated that a reduction in pH (from a mean of 6.21–5.53) reduced the frequency and numbers of staphylococci isolated from mature cheese, which confirms the findings of Dos Santos & Genigeorgis (11) that successful fermentation and rapid pH decrease are critical in the safety of cheese with regard to foodborne pathogens, and especially *Staph. aureus*. We consider that monitoring pH during production is simple enough to be recommended to all cheese producers.

Nonetheless, staphylococci may be present in freshly produced cheese in numbers large enough to produce enterotoxin yet be detected in only small numbers at the retail stage with a normal course of ripening (12). Until recently the methods for examining samples for staphylococcal enterotoxins have been technically difficult and comparatively expensive. Therefore we would recommend the use of the thermonuclease test (3) for the screening of samples in any large-scale investigation.

It is only in recent years that goat and sheep milk have been produced in commercial quantities in Britain, and indeed dairy legislation in both Scotland and in England and Wales refers only to cow milk. Other milks are subject to general food hygiene regulations, but these do not specify particular bacteriological standards for testing of milk, cheese or yoghurt, nor is there detailed legislative control from the point of production to the point of sale. Codes of practice for goat and sheep milk production have been published (13, 14) but have the disadvantage of placing an undue responsibility on local authorities for inspecting premises, and upon local health boards for proving that food epidemiologically associated with illness has been produced under unhygienic (but unspecified) conditions. In Canada Collins-Thompson and colleagues (4) have recommended bacteriological standards for cheese which depends upon the detection (in cheese at 60 days) of unacceptably large numbers of viable bacteria, particularly *E. coli* 1 and *Staph. aureus* (4). *Staph. aureus*, however, causes illness because of a toxin which persists, and this factor has not been addressed in proposals so far published, presumably because of the infrequent reporting of such an association. There has been a significant increase in the rate at which reports have appeared recently, notably in Czechoslovakia and France (5, 15). *Staph. aureus* was not detected in the cheese we tested from the 1984 production at the time of retail, though it was shown to have existed during production by the presence of enterotoxin. This was the first time that enterotoxin was detected in a food, associated with poisoning, produced in the UK, and from which viable *Staph. aureus* could not be isolated.

Warren & Arends demonstrated that staphylococci could be transmitted between cows via milking machines (16). The same problem might be anticipated with sheep. Contamination of milk and milk products is common (4-6, 9, 12, 15, 17-20), so the onus must be upon the industry and the producers to prove that they can maintain satisfactory standards.

E. coli was not a contaminant found in milk randomly tested prior to 1985 yet large numbers were present in the cheese produced during 1984. The cheesemaker had propagated a commercial culture by simply incubating this with raw milk, with no check on the purity of the result; furthermore, the dilution of this 'starter' was arbitrary. *E. coli* was found in large numbers on 2 May 1985 (in the absence of *Staph. aureus*), and as a result this 'starter' was abandoned in favour of a freeze-dried commercial culture supplied in a 'ready to use' sachet (Christian Hansen Laboratories, Reading). *E. coli* was not detected in cheese produced after that date, is rarely implicated as a cause of food poisoning and is tested usually only as an indicator of poor hygiene.

We have confirmed that coliforms reduce in numbers on ripening (21). However, Brodsky reported the isolation of *Salmonella muenster* from a 60-day-aged raw

milk cheese sample which had only 90 coliforms per gram of cheese (22). We have also demonstrated that a similar reduction in numbers of *Staph. aureus* occurs (Table 2), although as shown by this outbreak the toxin remains active. Consequently although the bacteriological standards suggested by Collins-Thompson and colleagues (4) might be applied to the freshly produced cheese, they would be inadequate for cheese at the time of retail, as they do not guarantee the absence of pathogenic organisms.

The hygienic production of goat milk (23) can be achieved by following Codes of Practice but they have no statutory force. A complaint of illness requires that the District Authorities have reasonable grounds to suspect that a food is likely to cause food poisoning', leaving the authorities with the burden of proof without defining the bacteriological parameters on which to base that suspicion. Consequently we tested hundreds of samples which should have been subject to quality control at the dairy.

The most effective way of eliminating the risk of food poisoning from milk and milk products is by heat treatment. There are however, particular difficulties facing producers of goat and sheep milk. Pasteurization would be expensive for farmers whose herds and flocks are small, yet not to insist on heat treatment might place them at a commercial advantage compared with cow-milk producers. Compulsory heat treatment of cow milk has been shown to significantly reduce the risk of community outbreaks in Scotland since 1983, particularly of salmonellosis and campylobacter infection (24). Action applied to other non-human mammalian milk might be expected to give similar health and cost benefits. *Staph. aureus* strains which are often enterotoxigenic are commonly associated with mastitis (6), and such organisms are reliably destroyed by pasteurization (18). The equipment for pasteurization of small batches of sheep milk need not be elaborate, but when large quantities of milk are handled the purchase of equipment for the High-Temperature-Short-Term (HTST) method of pasteurization may be justified. In contrast to cheese, yoghurt is always produced with heat-treated milk and yet is considered a 'natural' food, but heat treatment of milk prior to cheese production is said to be unacceptable to the consumer. The same arguments were used prior to compulsory heat treatment of cow milk. After the public health success of that measure in Scotland, and in the face of mounting evidence of food poisoning due to milk products from goats and sheep, we urge that these arguments be rejected and that legislation be introduced to ensure that all non-human mammalian milk and milk products be subject to the same legislation.

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