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The source of *Yersinia* spp. in pasteurized milk: an investigation at a dairy

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SUMMARY

Pasteurized bottled milk supplied by a single dairy was frequently found to be contaminated with *Yersinia* spp. Investigations were carried out at the dairy in an effort to pinpoint the source of these organisms. Viable counts obtained from milk bottle rinses indicated that bottle washing was often unsatisfactory, and on one occasion *Y. frederiksenii* was isolated from the pooled rinse water of six bottles. Samples of milk were taken on arrival at the dairy and at various stages following pasteurization. Heat resistance tests carried out on strains of yersinia isolated from pasteurized milk indicated that they would not survive the pasteurization process. However two strains of yersinia were isolated from a sample of milk taken immediately after pasteurization but before bottling. The thermograph indicated that the time/temperature conditions applied during pasteurization were adequate. The presence of yersinia strains in the milk at this stage therefore suggests that undetectable levels of raw milk were being allowed to contaminate the pasteurized milk. The absence of yersinia in cartoned samples produced on the same day as contaminated bottled samples indicated that environmental contamination of the bottle filler valve also may have occurred at times. Results of this investigation indicate that increased vigilance is required to ensure proper operation of pasteurizers and bottle washers.

INTRODUCTION

Recent studies have shown the common occurrence of *Yersinia* spp. in pasteurized milk from a number of dairies [1, 2]. Heat resistance studies [3] have indicated that strains of yersinia are unable to survive pasteurization, suggesting that their presence in pasteurized milk is either due to post-pasteurization contamination or under-processing. *Y. enterocolitica* can multiply at temperatures as low as 3 °C [4], and thus its presence in pasteurized milk, which is generally stored at refrigeration temperatures at the dairy, in the retail chain, and in the home, may be of public health significance.

Contaminated pasteurized milk was implicated in two episodes of yersinia infection which occurred amongst the patients in a paediatric ward of a district general hospital [5]. In an attempt to establish the source of the yersinia

organisms, the milk supplied to the ward was sampled, and the dairy supplying the hospital with pasteurized milk was investigated.

MATERIALS AND METHODS

Pasteurization and cleaning at the dairy

Raw milk is collected by tanker from six farms, and delivered to the dairy at about midday, where it is pumped into an insulated holding tank on the roof. The following day the milk is pasteurized by the high temperature, short time (HTST) process at 72·5 °C (162·5 °F) for 15 s. The pasteurizer was manufactured in 1981, and is normally set at a flow rate of 600 gallons per hour; 1500 gallons are pasteurized per day. At the end of each pasteurization run 'in-place' cleaning is carried out, the holding tanks and pipelines having a cold wash with mains water containing 1% of a chlorine-based detergent sanitizer. Removable parts of the bottle and carton filling equipment are washed and soaked in sanitizer. Once a week the holding tanks are dosed with a 1% concentration of a sodium hydroxide-based detergent at a high temperature to remove fat.

Milk bottles are washed in lines of 12 in a bottle washer also made in 1981. Bottles are pre-rinsed in water, and the water run to waste. The bottles then enter the detergent tank, which contains mains water at a temperature of 62·8 °C (145 °F) and a sodium hydroxide-based detergent. The bottles remain in this tank for 5 min and are then moved into the final rinse tank, through which water heated to 37·8 °C (100 °F) flows at a rate of 10 gallons per min. The bottle-washing plant is washed weekly with a chlorine-based sanitizer, the detergent tank emptied and scrubbed out once a week and the final rinse tank emptied and cleaned every 4 weeks. A diagram of the dairy is shown in Fig. 1.

Sampling

Samples of bottled pasteurized milk supplied by the dairy were obtained from the paediatric ward and from the pathology rest room of the hospital, and one-pint cartons of pasteurized milk were purchased from a local retail outlet. Sampling was carried out up to three times per week for 3 months, and occasional samples were obtained from the ward thereafter. Three samples from three-gallon cartons of homogenized, pasteurized milk supplied to other wards were also obtained.

The dairy was first visited 1 month after sampling began. Unfortunately pasteurization had been completed and cleaning had commenced. Swabs were taken from the empty tanks for raw milk, pasteurized milk, and homogenized and skimmed milk, and from the bottle filler valve and carton filling valve which were both soaking in the final chlorinated rinse after washing. Samples of tap water and chlorinated wash waters from bottle rinse tank, the separator, and the bottle unit parts were taken. An empty clean, capped, milk bottle, a one-pint carton of pasteurized milk, and a sample of raw milk delivered that day were also taken for testing.

Over a period of 2 months following this visit, members of the Environmental Health Department took samples of pasteurized milk from the nozzle of the bottle filler immediately before bottling, and samples of the final rinse water in the bottle washer, using sterile glass jars. They also submitted empty, clean milk bottles

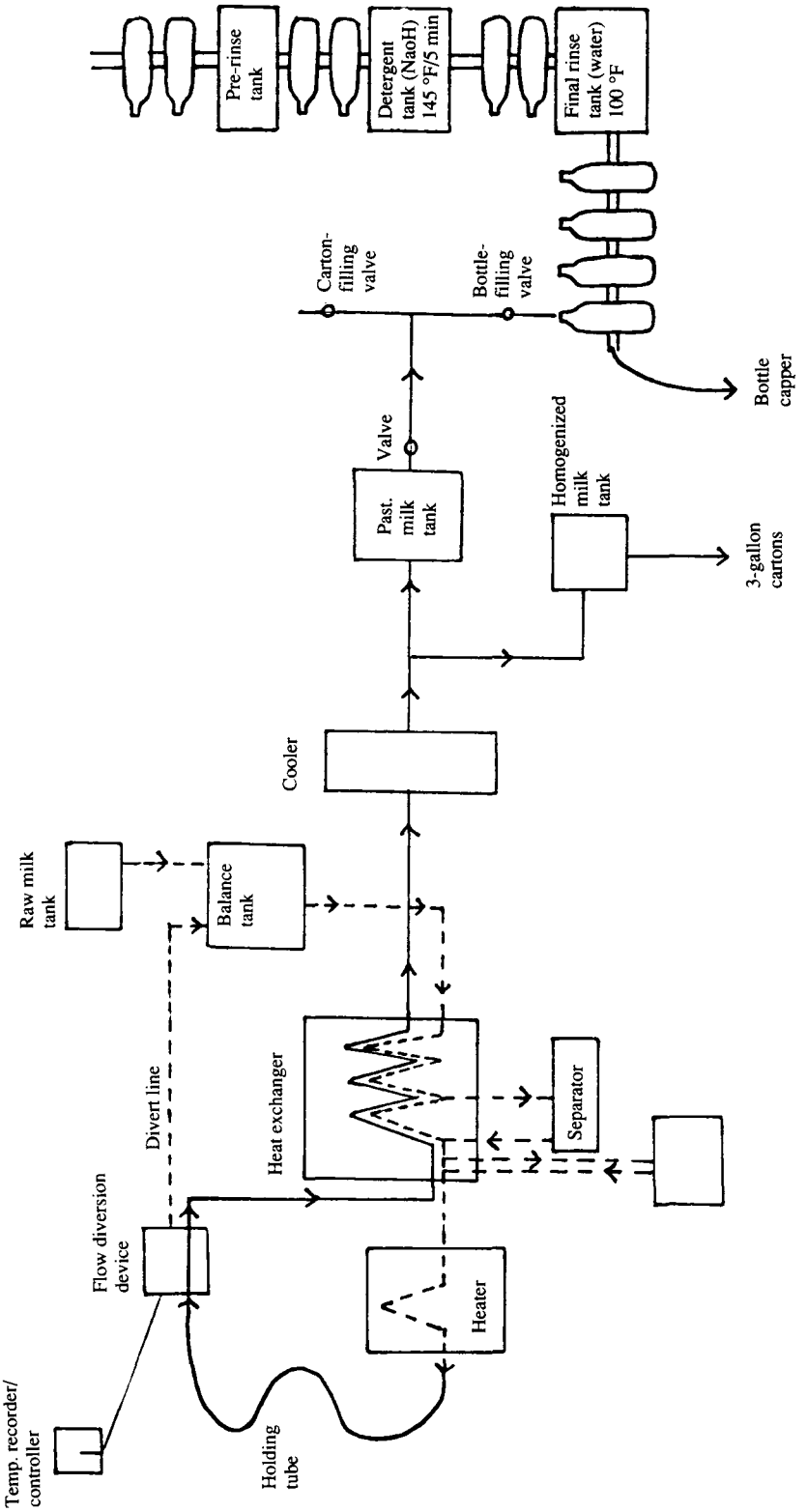

Fig. 1. Diagrammatic representation of the dairy plant. – – – >, flow of raw milk; ——>, flow of pasteurized milk.

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ready for filling but capped for transport, and samples of raw milk and skimmed, pasteurized bottled milk ready for retail.

A further visit was made to the dairy 6 months later to take samples during the monthly clean-out of the bottle washer. As the bottle washer was emptied, samples of the rinse water and sludge from both tanks were taken. Raw milk from each of the six farms supplying the dairy with milk for pasteurization that day was obtained. A sample of pasteurized milk taken into a sterile glass jar from the nozzle of the bottle filler immediately before bottling and a clean, capped milk bottle were also examined.

Following the second visit, milk bottles from the start, middle and end of the bottle-cleaning run were submitted every week by the Environmental Health Department. In addition, samples of the same milk after pasteurization but before bottling were obtained from the bottle filling valve. Bottled and cartoned milk samples were also sent for testing. Samples of pasteurized milk were taken from the tank above the bottling plant and from the filler nozzle at the beginning, middle and end of the run. This monitoring continued for 10 months.

Microbiological testing

Milk samples

Milk samples were examined for the presence of yersinia by two methods in parallel:

(1) 25 ml was added to 225 ml buffered peptone water, pH 7·3 (BPW; Oxoid) and then incubated at 4 °C for up to 3 weeks [1];
(2) 25 ml was added to 225 ml tris-buffered 1% peptone water, pH 8·0 (TPW) [6], and incubated at 9 °C for up to 3 weeks.

Both enrichment broths were subcultured to Cefsulodin Irgasan Novobiocin agar (CIN; Gibco) at weekly intervals for 3 weeks. Suspect colonies obtained on CIN agar after incubation for 20–24 h at 30 °C were tested by methods previously described [1]. Presumptive strains of yersinia were sent to the reference facility at the Public Health Laboratory, Leicester for confirmation and biotyping according to Bercovier and Mollaret [7], and serotyping by Wauters' scheme [8, 9].

Milk bottles

Twenty millilitres of quarter-strength Ringer's solution containing 0·05% sodium thiosulphate were introduced into each bottle, and the bottle rolled to allow thorough wetting of all surfaces. The Ringer's solution was left in contact with the bottle for 20 min. Two 5 ml aliquots of this rinse water were then placed in Petri dishes and 10 ml of molten, cooled milk agar (Oxoid) added to each plate [10]. After setting, one plate was incubated at 37 °C for 48 h and the other at 22 °C for 72 h. The number of colonies per plate was counted and the count per bottle calculated for each temperature. Counts of < 200/bottle were deemed satisfactory, 200–600/bottle fairly satisfactory, and > 600/bottle unsatisfactory [8]. In addition 5 ml aliquots of the rinse water were added to 45 ml volumes of BPW and TPW, which were incubated and examined for the presence of yersinia as described above.

Table 1. *Strains of* Yersinia *spp. examined for heat resistance*

No.	Strain	Biotype	Serotype	Source
1	*Y. enterocolitica*	1	10K	Pasteurized milk
2	*Y. intermedia*	—	NT*	Pasteurized milk
3	*Y. frederiksenii*	—	NT	Pasteurized milk
4	*Y. enterocolitica*	1	7	Pasteurized milk
5	*Y. enterocolitica*	1	6,30	Pasteurized milk
6	*Y. enterocolitica*	1	10K	Faeces, human
7	*Y. enterocolitica*	1	6,30	Faeces, human
8	*Y. frederiksenii*	—	NT	Faeces, human
9	*Y. enterocolitica*	1	7	Faeces, human
10	*Y. enterocolitica*	1	NT	Liquid pasteurized milk
11	*Y enterocolitica*	1	34	Raw beefburgers
12	*Y. enterocolitica*	1	NT	Smoked mackerel pâté
13	*Y. enterocolitica*	1	6,30	Frozen whole prawns
14	*Y. enterocolitica*	1	5,27	Raw chicken livers
15	*Y. kristensenii*	—	NT	Raw beef sausage
16	*Y. intermedia*	—	NT	Porcine throat swab
17	*Y. enterocolitica*	1	34	Ice cream
18	*Y. enterocolitica*	4	3	Faeces, human
19	*Y. enterocolitica*	3	NT	Faeces, human
20	*Y. kristensenii*	—	NT	Faeces, human
21	*Y. enterocolitica*	1	5,27	Faeces, human
22	*Y. enterocolitica*	1	5,27	Vanilla cream slice
23	*Y. enterocolitica*	1	5,27	Pasteurized milk
24	*Y. enterocolitica*	1	5,27	Porcine rectal swab
25	*Y. enterocolitica*	1	7	Frozen whole prawns
26	*Y. intermedia*	—	NT	Faeces, human

Strains 1–5, milk derived from dairy under investigation.
Strains 6–8, faecal samples derived from patients in paediatric ward of hospital.
* NT, not typable.

Heat resistance tests

Five different strains of yersinia isolated from the milk, 3 strains isolated from patients on the ward, and 18 other strains isolated from other milk, food and human sources were examined. The isolations are summarized in Table 1. Initial tests carried out at 55 °C identified six strains (numbers 1, 9, 10, 12, 22 and 23) which appeared to survive heating at this temperature for 15 min. Three of these strains (1, 22 and 23) and two others (6 and 21) were selected for further tests at 62 °C. These strains were grown at 30 °C for 48 h in buffered peptone water, and then diluted 1:100 in pasteurized milk. Ampoules containing 0·2 ml of the inoculated milk were then heated in a waterbath at 62 °C, At 5 min intervals for 35 min, one ampoule of each strain was removed and plunged into an icebath. Counts were then performed on horse blood agar, and colonies subcultured onto CIN agar for confirmation of identity. This procedure was repeated four times for each strain. The pasteurized milk used in these tests was also cultured by enrichment to ensure absence of *Yersinia* spp.

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Table 2. *Summary of yersinia isolations*

Sample	Origin of sample	*Strains of yersinia* Biotype	Sero-type	No. of isolations*
Past. bottled milk, 15/112 samples	Ward + rest room	*frederiksenii*	NT	10
		intermedia	NT	7
		enterocolitica 1	10K	4
		enterocolitica 1	7	1
Raw milk 4/8 samples	On arrival at dairy	*enterocolitica* 1	6,30	1
		enterocolitica 1	13,14	3
		frederiksenii	NT	2
Past. bottled milk, 7/35 samples	Ward	*frederiksenii*	NT	6
		intermedia	NT	2
		enterocolitica	NS	1
Raw milk 6/6 samples	Farms supplying dairy	*intermedia*	NT	4
		enterocolitica 1	13,14	1
		enterocolitica 1	6,30	1
Past. milk before bottling 1 sample	Dairy	*intermedia*	NT	1
		enterocolitica 1	13,14	1
Raw milk 7/9 samples	On arrival at dairy	*intermedia*	NT	6
		enterocolitica 1	5,27	2
		enterocolitica 1	13,14	1
Clean milk bottles (6 pooled)	Dairy	*frederiksenii*	NT	1

*Multiple strains from some samples.
NT, not typable.
NS, not serotyped.

RESULTS

Twenty-two strains of yersinia were isolated from 15 of 112 samples of bottled pasteurized milk obtained from the ward and rest room in the first 3 months of the survey (Table 2). Two different strains were isolated from four samples, and four different strains were isolated from one sample. Strains isolated were *Y. frederiksenii* serologically not typable (10), *Y. intermedia* serologically not typable (7), *Y. enterocolitica* biotype 1, serotype O.10K (4) and serotype O.7 (1). *Yersinia* spp. were detected in 13 of 43 batches of milk tested. No isolations of yersinia were made from 34 one-pint cartons of pasteurized milk tested during this period.

Over the following 14 months a further 35 samples of bottled pasteurized milk were obtained from the ward, 7 of which contained *Yersinia* spp. Five samples contained *Y. frederiksenii* serologically untypable, one sample contained untypable strains of *Y. frederiksenii* and *Y. intermedia*, and one sample contained untypable *Y. intermedia* and *Y. enterocolitica* biotype 1 (not serotyped). Samples taken from three-gallon cartons from other wards were not found to contain yersinia organisms.

Approximately 2 weeks before the first visit to the dairy, as a result of discussions with the Environmental Health Department, the dairy manager instigated the daily use of a chlorine-based sterilant in the bottle washer, which was allowed to stand overnight and then rinsed out before use. Not surprisingly

Table 3. *Recovery of yersinia organisms from inoculated milk heated to 62 °C*

Strain	Heating time (mins)	Yersinia/ml Test no. 1	2	3	4
1	0	$1{\cdot}1 \times 10^8$	$2{\cdot}7 \times 10^7$	$1{\cdot}6 \times 10^6$	$7{\cdot}0 \times 10^6$
	5	$1{\cdot}5 \times 10^2$	< 5*	$3{\cdot}3 \times 10^2$	< 5
	> 10	< 5	< 5	< 5	< 5
6	0	$9{\cdot}7 \times 10^7$	$2{\cdot}0 \times 10^7$	$5{\cdot}0 \times 10^6$	$1{\cdot}5 \times 10^6$
	5	$1{\cdot}5 \times 10^2$	< 5	< 5	< 5
	> 10	< 5	< 5	< 5	< 5
21	0	$6{\cdot}6 \times 10^6$	$2{\cdot}0 \times 10^7$	$8{\cdot}3 \times 10^6$	$5{\cdot}3 \times 10^6$
	5	$1{\cdot}6 \times 10^4$	< 5	< 5	< 5
	> 10	< 5	< 5	< 5	< 5
22	0	$5{\cdot}0 \times 10^6$	$3{\cdot}3 \times 10^6$	$5{\cdot}0 \times 10^6$	$2{\cdot}9 \times 10^6$
	5	$1{\cdot}6 \times 10^4$	< 5	< 5	< 5
	> 10	< 5	< 5	< 5	< 5
23	0	$3{\cdot}3 \times 10^6$	$1{\cdot}0 \times 10^7$	$5{\cdot}0 \times 10^6$	$4{\cdot}9 \times 10^6$
	5	$1{\cdot}5 \times 10^3$	< 5	< 5	< 5
	> 10	< 5	< 5	< 5	< 5

* < 5, limit of detection = 5/ml (contents of ampoule = 0·2 ml).

yersinia was not isolated from any sample taken on this visit, except for a strain of untypable *Y. frederiksenii* isolated from the raw milk.

Over the next 3 months, six strains of yersinia were isolated from four samples of raw milk: *Y. enterocolitica* biotype 1 serotypes O.6,30 (1 sample) and O.13,14 (3 samples), and *Y. intermedia* serologically untypable (2 samples). Yersinia was not isolated from any of 12 pasteurized milk samples taken immediately after pasteurization, but 1 of 7 skimmed pasteurized bottled milk samples taken at the same time was found to contain *Y. intermedia* (untypable). None of five samples of final rinse water was found to contain yersinia. Twenty-seven clean milk bottles were examined, but yersinia was not detected in any bottle. However 10 bottles had unsatisfactory rinse counts, with 5 counts exceeding 10^4 colony-forming units per bottle.

As a result of these poor bottle counts, the temperature of the warm water rinse was increased from 62·8 °C (145 °F) to 65·6 °C (150 °F), the detergent tank emptied and cleaned daily and boiled weekly, and the final rinse tank boiled every 4 weeks before emptying. An add-on chlorinator was brought into use which enabled chlorination of the final rinse water to 2–3 p.p.m. Two jets of chlorinated water lasting 10 s each delivered approximately one pint of water to the inside of each bottle. Initially counts obtained from bottle rinses improved slightly, with only 5 of 51 unsatisfactory and a further 3 fairly satisfactory results. However results from bottles examined 7–9 months later produced 18 unsatisfactory and 4 fairly satisfactory results from 30 bottles, and on one occasion *Y. frederiksenii* (untypable) was isolated from the pooled rinse water of 6 bottles.

All the samples of raw milk obtained from the six farms supplying the milk for pasteurization on the day of the second visit contained *Yersinia* spp. *Y. intermedia* (untypable) was isolated from four samples and *Y. enterocolitica* biotype 1,

serotypes O.6,30 and O.13,14 were isolated from the two remaining samples. The sample of pasteurized milk, taken from the bottle filling valve into a sterile glass jar immediately after pasteurization but before bottling, also contained *Y. intermedia* (untypable) and *Y. enterocolitica* serotype O.13,14. The samples taken from the rinse water and sludge in the tanks of the bottle washer and the clean, capped milk bottle were found to be free of yersinia contamination.

Nine strains of yersinia were isolated from 7 out of 9 samples of raw milk submitted in the following month: *Y. enterocolitica* biotype 1, serotypes O.5,27 (2) and O.13,14 (1), and *Y. intermedia* serologically untypable (6). *Yersinia* spp. were not isolated from any sample of milk taken during the next 9 months after pasteurization but before bottling, although isolations were made from 4 of 23 bottled milk samples obtained from the ward. A summary of all yersinia isolations is shown in Table 2.

Results of the heat resistance tests are shown in Table 3. No strain could be recovered after 10 min heat treatment at 62 °C, despite high initial inocula of 10^6–10^8 c.f.u./ml. Four strains could only be recovered in 1 of 4 tests after 5 min heat treatment, and the fifth strain was recovered from 2 of 4 tests.

DISCUSSION

The occurrence of *Yersinia* spp. in pasteurized milk supplied by this dairy appeared to be quite common. Almost one-third of the batches of milk tested in the first 3 months were contaminated with yersinia, but these organisms were not isolated from every bottle tested from the same batch. This suggests that the number of contaminating yersinia organisms was quite low. Multiple strains of yersinia in a single sample of pasteurized milk also occurred frequently. The lack of isolations from cartoned milk from the dairy and from patients who had drunk cartoned milk [5] pointed to the bottles as a source of contamination, and indeed a strain of *Y. frederiksenii* was eventually isolated from the pooled bottle washings of six bottles supposedly clean and ready for filling. The poor colony counts frequently obtained from the milk bottles before the chlorinator was added to the final rinse tank of the bottle washer also supports the hypothesis that the bottles themselves were a source of yersinia contamination. Although bottle rinse results obtained after the introduction of the chlorinator improved, some poor results still occurred. Bottles are held upside down to drain in the bottle washer, and the contact time of the chlorinated water with the inside of the bottles is only short, about 20 s.

The samples of milk taken on the second visit to the dairy immediately after pasteurization contained two strains of yersinia identical to those found in the raw milk, an untypable strain of *Y. intermedia* and *Y. enterocolitica* serotype O.13,14. This latter strain is an unusual serotype, but had been isolated before by the laboratory from raw milk delivered to this dairy. The presence of these strains in the milk at the post-pasteurization stage suggests four alternatives: (1) Yersinia organisms can survive pasteurization; (2) The pasteurization temperature was not reached; (3) Raw milk was being allowed to get through the pasteurization process; (4) The post-pasteurization holding tank and pipework leading to the bottle filler were harbouring yersinia organisms.

Results of heat resistance tests carried out on five strains isolated from pasteurized milk supplied by this dairy and many other strains from other milk, food and human sources indicate that, like most other Enterobacteriaceae, yersinia are not unusually heat-resistant and should not be able to survive pasteurization temperatures. Hughes [11] however reported that six isolates of yersinia derived from pasteurized milk consistently survived pasteurization in milk containing approximately 10^8 organisms/ml. Lovett and co-workers [3] examined these strains and 42 other milk isolates for heat resistance at 62·8 °C. Three strains were found to be unusually heat resistant, with D values at this temperature of 0·24–0·96 min and z values of 5·11–5·78 °C. Further investigations showed that despite this heat resistance, none of these strains would be able to survive the pasteurization process. In theory, there is a possibility that yersinia organisms (and other coliforms) are not killed by the 15 s HTST pasteurization process but are only injured, and that the prolonged incubation at low temperatures employed for the isolation of yersinia allows recovery and growth of the stressed cells. Other workers have shown that *Listeria monocytogenes* may survive in this way [12]. This hypothesis needs further investigation.

Inspection of the thermographs by the Environmental Health Officer did not reveal any aberrations in the time/temperature conditions applied during pasteurization, and results of phosphatase testing carried out by the Public Health Laboratory Service had always been satisfactory. There was no evidence of leakage of raw milk through the system. The phosphatase test, which is relied upon to detect the presence of raw milk, is able to detect the presence of 0·2 % raw milk [13] in pasteurized milk. The possibility remains that small amounts of raw milk containing yersinia were being allowed to contaminate the pasteurized milk without being detected by the phosphatase test. After pasteurization, the milk passed to one holding tank for filling one-pint bottles and cartons, and a second holding tank for filling three-gallon milk packs. The pipework from the former tank led to a T-piece with one outlet to bottles and the other to cartons. The consistent isolation of yersinia from bottled and the lack of isolation from cartoned milk suggests that if there was post-pasteurization contamination of the plant, it would be localized in the T-piece outlet leading to the bottles. This seems rather unlikely, as this part of the equipment was apparently dismantled daily for washing and soaking in sanitizer, and also presupposes that one of the first three alternatives had also occurred. Whilst the bottle filling valve may have become contaminated from the environment, there was no evidence of leakage of raw milk in the dairy, but the strains isolated from the pasteurized sample taken from the bottle filling valve were identical to 2 of the 3 strains found in the raw milk being pasteurized that day. Results shown in Table 2 show that a variety of strains were obtained from both raw and pasteurized milk samples. In addition, milk delivered to the dairy could originate from any 6 of 60 farms, so that strains present in the raw milk might vary from day to day. The likelihood of the bottle filling valve being contaminated on this occasion by strains identical to those found in the raw milk but originating from the environment seems remote. However lack of isolation of yersinia from cartoned samples produced on the same day as contaminated bottled samples suggests that contamination of the bottle filler valve may have occurred at other times.

Although the source of yersinia organisms in the pasteurized milk was not conclusively identified in this investigation, it seems most probable that small amounts of raw or underpasteurized milk containing yersinia were being allowed through the system. Absence of yersinia in cartoned milk indicated that contamination of the bottle filling valve was occurring and poor bottle washing was also contributing to the regular presence of yersinia organisms in the bottled pasteurized milk supplied by this dairy. Improvements introduced during the course of the investigation and greater vigilance resulting from the visits to the dairy appeared to be effective in reducing yersinia contamination. Previous studies have indicated that the occasional use of steam during cleaning can help to eliminate yersinia contamination of the plant [1]. Increased efforts are required to ensure the proper operation of pasteurizers and bottle washers, with particular emphasis on elimination of possible leakage of raw milk and maintenance of positive pressure during pasteurization and stricter checks of temperature levels. The common occurrence of yersinia in pasteurized milk from other dairies [1] indicates that these precautions are applicable to all processing plants in the dairy industry.

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REFERENCES

1. Greenwood MH, Hooper WL. *Yersinia* spp. in foods related environments. Food Microbiol 1985; **2**: 263–9.
2. Walker SJ, Gilmour A. The incidence of *Yersinia enterocolitica* and *Yersinia enterocolitica*-like organisms in raw and pasteurized milk in Northern Ireland. J Appl Bacteriol 1986; **61**: 133–8.
3. Lovett J, Bradshaw JG, Peeler JT. Thermal inactivation of *Yersinia enterocolitica* in milk. Appl Environ Microbiol 1982; **44**: 517–19.
4. Stern NJ, Pierson MD, Kotula AW. Growth and competitive nature of *Yersinia enterocolitica* in whole milk. J Food Sci 1980; **45**: 972–4.
5. Greenwood MH, Hooper WL. Excretion of *Yersinia* spp. associated with consumption of pasteurized milk. Epidemiol Infect 1990; **104**: 345–50.
6. Greenwood MH, Hooper WL. The use of alkalinity and incubation at 9 °C for improved recovery of *Yersinia* spp. from faeces. Epidemiol Infect 1988, **101**: 53–8.
7. Bercovier H, Mollaret HH. *Yersinia*. In: Krieg NR, Holt JG, eds. Bergey's manual of systematic bacteriology, vol 1. Baltimore: Williams and Wilkins, 1984: 498–506.
8. Wauters G, Le Minor L, Chalon AM. Antigenes somatique et flagellaires des *Yersinia enterocolitica*. Ann Inst Pasteur 1971; **120**: 631–42.
9. Wauters G, Le Minor L, Chalon AM, Lassen J. Supplement au scheme antigenique de *Yersinia enterocolitica*. Ann Inst Pasteur 1972; **122**: 951–6.
10. Anon. The rinse method for the examination of washed milk bottles. Ministry of Agriculture and Fisheries 1974; Technique No. B743/T.P.B.
11. Hughes D. Isolation of *Yersinia enterocolitica* from milk and a dairy farm in Australia. J Appl Bacteriol 1979; **46**: 125–30.
12. Fernandez Garayzabal JF, Dominguez Rodriguez L, Vazquez Boland JA, Rodriguez Ferri EF, Briones Dieste V, Blanco Cancelo JL, Suarez Fernandez G. Survival of *Listeria monocytogenes* in raw milk treated in a pilot plant size pasteurizer. J Appl Bacteriol 1987: **63**: 533–7.
13. Anon. Pasteurizing Plant Manual. Society of Dairy Technology, 1983.