ACM/485

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

PRELIMINARY RESULTS FROM THE NATIONAL STUDY ON THE MICROBIOLOGICAL QUALITY AND HEAT PROCESSING OF COWS' MILK : *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* (MAP)*

1. The attached paper summarises the MAP results to date from the Food Standards Agency's national study on the microbiological quality and heat processing of cows' milk.

2. The results of other microbiological examinations carried out will be presented to the Committee at a future date.

3. The paper will be introduced by Ms Geraldine Hoad (FSA).

Secretariat
September 2000

* In previous ACMSF papers, MAP has been referred to as *Mycobacterium paratuberculosis* (MPTB).

PRELIMINARY RESULTS FROM THE NATIONAL STUDY ON THE MICROBIOLOGICAL QUALITY AND HEAT PROCESSING OF COWS MILK : *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

Background

1. The National Study on the Microbiological Quality and Heat Processing of Cows' Milk ran for 18 months, from March 1999 to August 2000. It comprised a representative sample of approved dairy establishments throughout the UK which heat treat milk (both drinking milk and milk for the manufacture of milk products). In addition to microbiological examination of samples of milk before and after heat processing, details of the quality management systems and process controls were collected.

2. The objectives of the study were:

- To obtain data on the microbiological quality of cows' milk in the UK, before and after heat processing.

- To obtain and analyse details of the production process by which the milk was heat treated.

3. At the request of the ACMSF, a proportion of samples are being examined for the presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), to examine the effectiveness of pasteurisation in destroying this organism. This paper summarises the MAP results available to date. A full set of results will not be with the Food Standards Agency until the end of the year. A final report detailing all the results of the survey will be published in 2001.

Study Design

4. All 755 approved dairy establishments in the UK who heat treat milk were invited to take part in the study and 258 dairies agreed to participate. Although participation was voluntary, every effort was made to ensure that the dairies sampled were as representative as possible of those in the UK as a whole in terms of size, location, etc.

5. Each dairy was asked to complete a form providing details about throughput, staffing levels, training, quality management and process controls, prior to the first sampling visit. At each sampling visit, samplers were also asked to complete a sampling form in consultation with the dairy. This recorded details

of the samples taken and the order of the processes which they have gone through e.g. separation, homogenisation.

6. The number of sampling visits a dairy received was determined by their size, i.e. litres of milk processed per annum (see Table 1). At each sampling visit, one raw and a number of heat treated milk samples (pasteurised whole/semi-skimmed/skimmed or UHT) were collected. Wherever possible, the raw and pasteurised milk samples originated from the same batch of milk.

Table 1: Number of sampling visits based on dairy size

Dairy Size (litres/annum)	Number of Visits
<100,000	1
100,000 - 500,000	2
500,000 - 1 million	2
1 million - 25 million	3
25 million - 50 million	3
50 million - 100 million	3
> 100 million	4

7. One set of samples (i.e. all the samples from a single visit) collected from each dairy participating in the survey were examined for the presence of MAP.

8. The methodology for MAP comprised a rapid screen, using immunomagnetic separation coupled to PCR, in conjunction with conventional decontamination and culture to confirm viability. When acid-fast cells were observed, further confirmatory tests were carried out. An acid-fast isolate was considered to be viable MAP only if it met **all** of the following criteria:

- Slow growth rate (around 4 weeks at 37°C before colonies are visible) and typical colony morphology (2-3 mm diameter, white) on Herrold's egg yolk medium slopes.
- Presence of IS900 insertion element confirmed by PCR.
- Dependence on mycobactin J for growth.

Results

9. A total of 830 samples of raw or pasteurised milk have been tested for the presence of MAP. Due to the very slow growth rate of this bacterium, some results are still pending but as of 31st July 2000, confirmed results are available for 679 (81.8%) of the samples tested (see Tables 2 and 3). Based on the available results, viable MAP has been found in 1.9% of raw milk samples and 2.1% of pasteurised milk samples.

Table 2: MAP Results to date - all samples

	Number of confirmed MAP results		
	Negative	Positive	Total
Raw milk (%)	197 (98.0)	4 (2.0)	201 (29.6)
Pasteurised milk (%)	466 (97.9)	10 (2.1)	476 (70.1)
UHT milk (%)	2 (100)	0	2 (0.3)
Total	665 (97.9)	14 (2.1)	679

Table 3: MAP Results to date - pasteurised samples

	Number of confirmed MAP results		
	Negative	Positive	Total
Pasteurised whole milk (%)	188 (98.4)	3 (1.6)	191 (40.1)
Pasteurised semi-skimmed milk (%)	140 (96.6)	5 (3.4)	145 (30.5)
Pasteurised skimmed milk (%)	138 (98.6)	2 (1.4)	140 (29.4)
Total	466 (97.9)	10 (2.1)	476

10. The 10 samples of pasteurised milk found to contain MAP were collected from eight different dairies, situated throughout the UK. The dairies varied in size from very small to very large, with outputs ranging from <100,000 to >100 million litres of heat treated milk per annum. The times and temperatures at which each of the MAP positive pasteurised milk samples were pasteurised are shown in Table 4. In the five cases where the raw milk sample and the pasteurised milk samples found to be positive came from the same batch of milk, the raw milk sample was negative.

Table 4: Time/Temperature at which MAP positive samples were pasteurised

Dairy	Sample type*	Temperature (°C)	Time (s)
1	PSS	72	14[#]
2	PSS	72	15
3	PW	72	15
4	PW + PS	72	15
5	PSS + PS	74	15
6	PSS	72.2	25
7	PW	74	25 – 26
8	PSS	75	25

*Sample types: P = pasteurised, W = whole, S = skimmed, SS = semi-skimmed
Sample passed phosphatase test and gave Fluorophos result of 59.8 mU/L

Follow up action

11. In all cases where pasteurised milk samples were found to contain MAP, follow up checks were carried out at the dairies. This was to ensure that the information collected by the samplers was correct and to confirm, as far as possible, that the milk had been correctly pasteurised and that no cross contamination had taken place following pasteurisation. All the investigations carried out to date have concluded that pasteurisation was carried out effectively and that post-pasteurisation contamination was very unlikely.

Molecular Typing

12. All the MAP isolates (from both raw and pasteurised milk) and the four laboratory strains in use during the survey are undergoing molecular typing using PFGE and RFLP techniques.

- PFGE typing is being carried out using three enzymes. To date, 7 of the 10 isolates from pasteurised milk and 3 of the 4 isolates from raw milk have been typed, as well the four laboratory strains. Results have shown five different PFGE types, with the majority of isolates being one of two types. However, two of the isolates, both of which came from pasteurised milk, each have a unique profile.
- RFLP typing using two restriction endonucleases has been carried out on all the isolates using a standardised protocol for the typing of MAP strains developed by Pavlik et al, 1999[1]. Results are shown in Table 5.

[1] Pavlik, I., Horvathova, A., Dvosdka, L., Bartl, J., Svastova, P., du Maine, R. and Rychlik, I. (1999) Standardisation of restriction fragment length polymorphism analysis for *Mycobacterium avium* subspecies *paratuberculosis*. Journal of Microbiological Methods 38, 155-167.

Table 5: Results of RFLP Typing

Dairy	Sample Type	RFLP type
1	PSS	C5
2	PSS	C1
3	PW	C5
4	PW + PS	C14 C16?[#]
5	PSS + PS	C5 C5
6	PSS	C1
7	PW	C5
8	PSS	C1

Laboratory strain	RFLP type
ACTC19698	C5
NCTC 8578	C5
B2	C5
DVL 943	*

The profile of this strain most closely resembles type C16 but does not correspond exactly to the published profile.
*The profile of DVL 943 does not coincide with any of the published profiles and is different from all the other strains isolated.

13. Thus it has been shown that five of the MAP isolates (those with RFLP types C1, C14 and C16?) from the survey are distinct from any of the laboratory strains and could not have arisen from cross contamination.

14. It should be noted that RFLP typing of 1008 MAP strains from all over the world by Pavlik *et al*., using the standardised protocol, has shown 28 different RFLP types, the majority of the strains (80.5%) being one of two types. Of the 47 strains of MAP which were from the UK, the majority (66%) were the same type.

Other results

15. The results of the other microbiological examinations carried out will be reported at a future meeting of the ACMSF.

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Microbiological Safety Division