MAP contamination and its removal from animal products

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- Current position ré MAP and human disease
- Review of evidence for MAP contamination in animal products
- Processing options to eliminate/inactivate MAP in milk

MAP and human disease

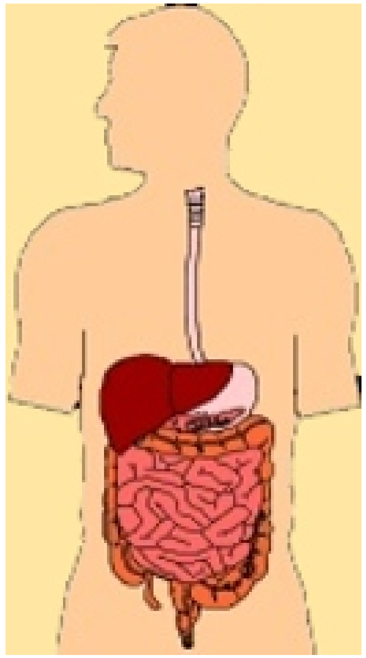
Long-time association with:

Crohn’s disease

Recently links suggested with:

Type 1 Diabetes mellitus

Irritable bowel syndrome



MAP and Crohn’s disease (CD)

Recent meta-analysis of published PCR and ELISA studies of association between MAP and Crohn’s disease - Feller et al. (2007) Lancet Infectious Diseases 7: 607-613:

“The association of MAP with CD seems to be specific, but its role in the aetiology of CD remains to be defined.”

Recent review of epidemiological data - Uzoigwe et al. (2007) Epidemiology and Infection 135: 1057-68:

“Epidemiologists have gathered enough information that points to an association between MAP and CD.”

Genetic predisposition in CD confirmed – NOD2 + at least six more genes - Massey and Parkes (2007), Gut 56: 1489-1492:

“Abnormalities in the handling of intracellular bacteria are emerging as a key theme in CD pathogenesis.”

Recent publications:

- NOD2 mutations in CD are associated with diminished mucosal antimicrobial peptide expression → ineffective clearance of intracellular MAP infection? - Peyrin-Biroulet et al. (2007)
- "Important role for autophagy in predisposing to CD" – Massey and Parkes (2007)
- "One might hypothesise that defective recognition of MAP cells in CD patients with NOD2 mutations is involved in pathogenesis of CD" - Ferwerde et al. (2007)
- Significantly higher TNF-α concentrations in gut mucosal organ culture supernatents from CD compared to UC, IBS and controls. "The data link MAP with a pathogenic mechanism in CD and is consistent with abnormal macrophage handling of MAP." – Clancy et al. (2007)

MAP and Irritable Bowel Syndrome (IBS)

Scanu et al. (2007) *Mycobacterium avium* subspecies *paratuberculosis* infection in cases of irritable bowel syndrome and comparison with Crohn's disease and Johne's disease: common neural and immune pathogenicities. Journal of Clinical Microbiology 45(12): in press

Mucosal biopsies tested PCR positive for MAP in:

15/20 (75%) IBS patients

20/23 (87%) CD patients

3/20 (15%) non-IBS/IBD controls

MAP and Type 1 Diabetes Mellitus (T1DM)

Dow, C.T. (2006) Paratuberculosis and Type I diabetes: Is this the trigger? Medical Hypotheses 67(4): 782-785

- Gene involved in regulation of the immune system (PTPN2) is present in both T1DM and CD
- Exposure to cow's milk early in life is a recognized risk factor in the development of T1DM

Sechi et al. (2007) Association of *Mycobacterium avium subsp. paratuberculosis* with Type-1 diabetes, a possible trigger. 9th International Colloquium on Paratuberculosis, Tsukuba, Japan

- IS900 PCR applied to blood (PMBC) of 46 Type 1 diabetic patients and 50 healthy controls:

  29/46 (63%) diabetic patients PCR positive for MAP

  8/50 (16%) control patients PCR positive for MAP

- Diabetic patients exhibited significant humoral immune responses to two recombinant MAP antigens (HbHA and GSD proteins) and the whole cell lysate of MAP, healthy controls did not

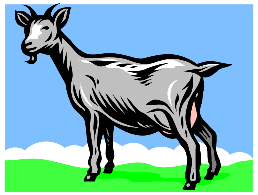
Food Surveillance

Raw cows' milk

Numerous studies documenting the culture and/or PCR detection of MAP in raw milk from:

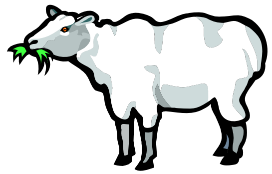
- Individual cows in a Johne's affected herd
- Bulk tank milk at farm level
- Bulk silo milk at processing level

Difficult to quantify the number of MAP present because of limitations in current cultural methods



Raw goats' milk

Country	% PCR positive	% culture positive	Reference
United Kingdom	1.1	0	Grant et al. (2001)
Norway	7.1	0	Djonne et al. (2003)
Switzerland	23.0	Not done	Muehlherr et al. (2003)



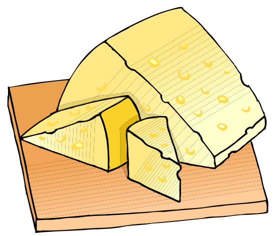
Raw sheep's milk

Country	% PCR positive	% culture positive	Reference
United Kingdom	0	0	Grant et al. (2001)
Switzerland	23.8	Not done	Muehlherr et al. (2003)

MAP in retail pasteurised cow's milk

Country	Size of survey	% PCR positive	% culture positive	Reference
England and Wales	312	7	0	Millar et al. (1996)
United Kingdom	567	11.8	1.8	Grant et al. (2002)
Ontario, Canada	710	15	0	Gao et al. (2002)
Republic of Ireland	357	9.8	0	O'Reilly et al. (2004)
Czech Republic	244	-	1.6	Ayele et al. (2005)
USA (CA, MN and WI)	702	64	2.8	Ellingson et al. (2005)
Argentina	70	Not done	2.9	Paolicchi et al. (2005)
Italy	22	4.5	0	Lillini et al. (2007)
India	43	38.8	72.2	Shankar et al. (2007)

MAP in retail cheese



Country	Type of cheese	Size of survey	Findings	Reference
Greece	Feta	42	50% PCR + 4.7% culture +	Ikonomopoulos et al. (2004)
Czech Republic	Hard and semi-hard	42	12% PCR + 2.4% culture +	
USA (WI and MN)	Not stated	98	5% PCR + No viable MAP isolated	Clark et al. (2006)
Switzerland	Soft, semi-hard and hard raw milk cheeses	143	4.2% PCR + No viable MAP isolated	Stephan et al. (2007)
Scotland	Artisanal farmhouse raw milk cheeses	5 (testing still in progress)	20% PCR + 40% suspect culture +	Williams and Withers (2007)

MAP in powdered infant milk formula

Country	Size of survey	% PCR positive	% culture positive	Reference
Czech Republic	51[a]	49	2.0	Hruska et al. (2005)
United Kingdom	45[b]	24.4	2.2*	Grant and Thompson (2007, unpublished)

[a] from 10 producers operating in 7 EU countries
[b] from 6 producers, all UK-based except one

* UK samples were also tested using *FASTPlaque*TB phage assay and two IMF samples yielded plaques indicating the presence of viable mycobacteria

MAP in spray-dried milk powder

Milk powders from three plants within N. Ireland surveyed over a 1 year period using IS900 PCR and culture

Country	Size of survey	% PCR positive	% culture positive	Reference
Northern Ireland	190	9.5	0	Rowe et al. (2007)

MAP in ground/minced beef

Country	Size of survey	% PCR positive	% culture positive	Reference
Republic of Ireland	113[a]	Not done	0	Egan et al. (2005)
USA (California)	200[b]	0	Not done	Jaravata et al. (2007)

[a] Minced beef sampled from single meat processing plant over 4 month period

[b] Ground beef purchased from three supermarkets in Sacramento over 3 month period

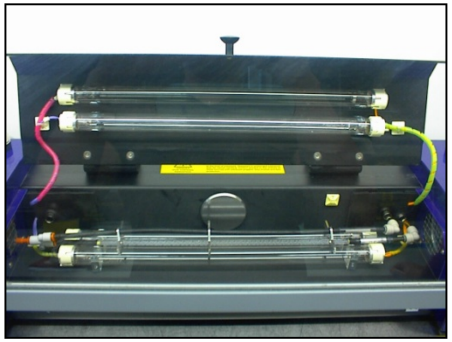
Current position ré MAP contamination of foods

- Detectable levels of viable MAP or MAP DNA in raw and pasteurised cows' milk, raw sheep and goats' milk, raw milk cheeses and infant milk formulae
- No evidence of MAP contamination of ground/minced beef
- Is this a true picture? - limitations of current detection methods particularly culture of MAP

Pasteurisation

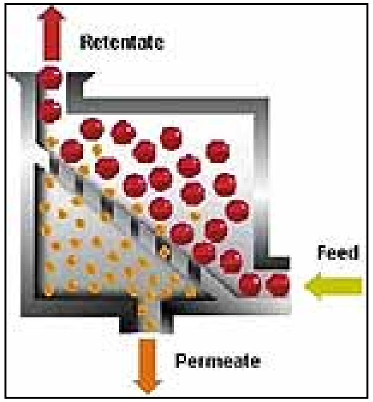
Centrifugation

Milk Processing Options



UV treatment?

Homogenisation



Microfiltration

Efficacy of HTST pasteurisation to inactivate MAP

Approach taken	MAP survival observed
Laboratory studies	**Yes** in 7 of 9 studies
Commercial-scale pasteurisation of naturally infected milk	**Yes** in 2 of 2 studies
Pilot/commercial-scale pasteurisation of spiked milk	**Yes** in 4 of 6 studies
Surveillance of commercially pasteurised milk	**Yes** in 5 of 9 studies

18 of 26 studies report potential survival of low numbers of viable MAP in milk after pasteurisation

Findings of DEFRA LINK MAP project

Grant et al. (2005) Applied and Environmental Microbiology 71(6), 2853-2861
Grant et al. (2005) International Journal of Dairy Technology 58(3), 138-142

- **Adjustments to pasteurisation temperature or holding time alone will not necessarily ensure complete inactivation of MAP**
  - low numbers of surviving MAP isolated from milks heat treated at temperatures from 72.5-82.5^{o}C
  - 25 s hold time found to be no more effective than 15 s hold time
- **Results indicated that pasteurisation needed to be combined with a process such as homogenisation in order to maximise the log kill**
  - heat treatments incorporating in-hold and, under certain circumstances, upstream homogenisation resulted in significantly fewer milk samples testing culture positive for MAP

Homogenisation and MAP

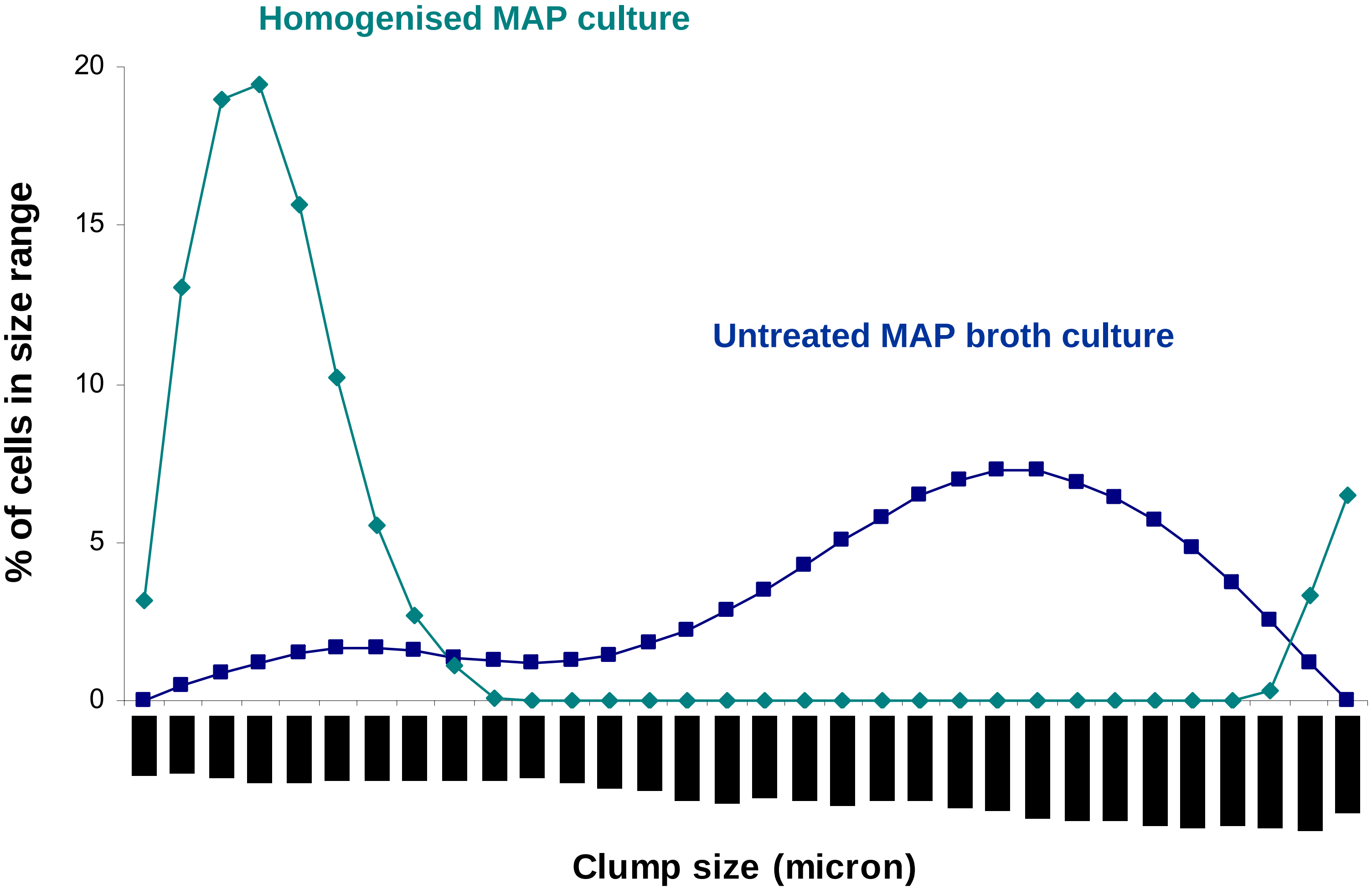
Milk is forced through a tiny orifice under considerable pressure in order to reduce size of fat globules

Additional effect of homogenisation is disruption of MAP clumps

Evidence:

- A 1 log_{10} increase in numbers of MAP after homogenisation at 27,000 kg/cm^2 – McDonald et al. 2005
- Cell size distribution profiles of control and homogenised MAP broth cultures – Grant et al. 2005

Homogenisation and MAP



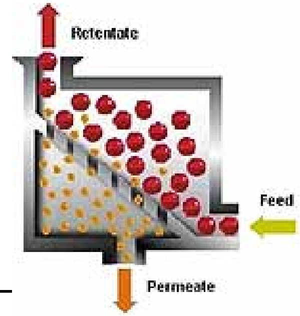
Centrifugation and MAP

- Commercial conditions for bactofugation: 7,000 x g for 10s applied to pre-heated milk (60^{o}C)
- Impact of these conditions on two MAP strains (NCTC 8578 and B4) in spiked milk was assessed

De-clumped cells	–	**85-93% removal**
Clumped cells	–	**74-79% removal**

- Clumps of MAP cells were not as readily removed from milk as single MAP cells by centrifugal force (p=0.0532 and p=0.0829)

Microfiltration and MAP



- Filtered milk is produced commercially: PurFiltre™
- 1.4 μm pore size used in commercial practice for skim milk

Impact of microfiltration (pore size: 1.2 μm) on MAP broth cultures was assessed

Observed effect	MAP strain						
	ATCC 19698	NCTC 8578	B4	DVL 943	806R	806PSS	377PW
Mean Log_{10} reduction	2.9	3.4	1.3	2.5	1.8	2.4	2.1
Mean % reduction	99.8	99.9	94.6	99.6	97.9	99.6	98.1

- Microfiltration can only be applied to skim milk, so MAP in cream fraction will still need to be inactivated by heat treatment

Impact of UV on MAP in milk

Altic, Rowe and Grant (2007) UV light inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk as assessed by *FASTPlaque*TB phage assay and culture. Appl. Environ. Microbiol. 73(11): 3728-3733

- MAP is more resistant to UV light than other milk microorganisms studied to date.
- Only a 0.5-1.0 $\log_{10}$ reduction of MAP in whole or semi-skim milk would be achieved before unacceptable organoleptic changes occur
- UV treatment is not a viable alternative to HTST pasteurisation to reduce numbers of MAP in milk

Current position ré milk processing options

- There has been a considerable amount of research on the impact of various dairy processes on MAP in milk
- HTST pasteurisation of milk does not achieve 100% inactivation of MAP 100% of the time
- Processes such as homogenisation, centrifugation and microfiltration could assist inactivation or removal of MAP in milk
- UV treatment of milk does not achieve a significant reduction in numbers of viable MAP