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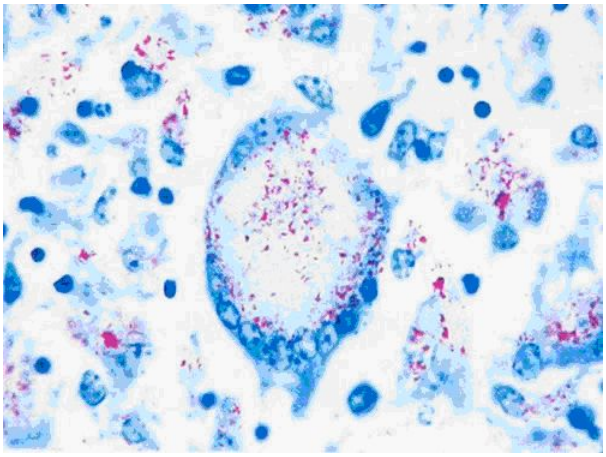
Survival of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in a raw milk smear type cheese



Key external stakeholders:
Artisanal farmhouse cheese producers; dairy industry; dairy farmers

Practical implications for stakeholders:
Johne's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and affects cattle, sheep and goats. Because of the similarity of the pathogenesis of Johne's disease in cattle and Crohn's disease in humans there is ongoing debate regarding the potential of animal derived MAP in the food chain to cause Crohn's disease in humans However, this link has never been definitively established.

The main recommendation from this research is that milk from cows suffering from Johne's disease and shedding large numbers of MAP should not be used for the manufacture of smear type cheese made from unpasteurised milk as these bacteria will survive cheese manufacture and ripening.

Main results:
To establish the fate of MAP in a raw milk smear type cheese the survival of MAP in a smear type cheese made from raw milk and the effect of the natural antimicrobial lacticin 3147 on the survival of MAP were assessed during manufacture and ripening.

- MAP can survive the manufacturing and ripening conditions employed in the making of a raw milk smear type cheese when the milk is artificially contaminated before cheese manufacture.
- The use of a lacticin 3147 producing starter did not affect MAP numbers after 4 weeks of ripening when compared to the control.

Opportunity / Benefit:
These results show that raw milk from cows suffering from Johne's disease and shedding *Mycobacterium avium* subspecies *paratuberculosis* should not enter the food chain if it is to be used to make unpasteurised smear type cheese.

Collaborating Institutions:
See page two of the full Technology Update

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The full list of the project collaborators in the other theme areas is available on http://www.vigilanciasanitaria.es/paratbtools/partners.php

1. Project background:

Johne's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and affects cattle sheep and goats. Because of the similarity of the pathogenesis of Johne's disease in cattle and Crohn's disease in humans there is ongoing debate regarding the potential of animal derived MAP in the food chain to cause Crohn's disease in humans. However, this link has never been definitively established. A review published in 2009 by the Food Safety Authority of Ireland (SFAI) of the available literature and opinion published between 2000 and 2008 concluded that current evidence did not support a causal relationship between MAP and the incidence of Crohn's disease. MAP bacteria grow and multiply inside the cells of the animal's immune system and are excreted by the infected animal in their faeces and to a lesser extent in the milk and saliva. While MAP does not grow in milk it can be present in the milk of affected animals and can also survive for long periods in the environment due to its tolerance to cold, drying and heat. Therefore the potential exists for milk contaminated by MAP to enter the food chain. In the early part of this century concerns arose that MAP cells if present in unpasteurised milk may survive pasteurization. However, work in Teagasc Food Research Centre, Moorepark and elsewhere have confirmed that MAP cells do not survive normal pasteurization processes. However, if MAP were present in unpasteurised milk concerns exist that it may survive the manufacture and ripening of cheeses particularly, those with a short shelf life.
In 2007 the EU funded a large project entitled Para TB Tools under FP6 comprising of 5 theme areas in collaboration with 27 partners in Europe, the US and Argentina. The theme areas were 1) Diagnostic tools; 2) Host Pathogen Interactions; 3) Food Safety; 4) Risk and Control and 5) Crohn's Disease. Teagasc's input was in the theme area of Food Safety.

2. Questions addressed by the project:

- Does MAP survive manufacture and ripening of a smear ripened cheese made from unpasteurised milk?
- Does the bacteriocin producing strain Lactococcus lactis DPC 3147, when used as a starter culture in the manufacture of smear ripened cheese made with unpasteurised milk, impact on the survival of MAP during ripening?

3. The experimental studies:

Two 10L vats of unpasteurised milk were artificially contaminated with ~10^4 *Mycobacterium avium* subspecies *paratuberculosis* ATTC *19698*/ml milk. Two cheeses were manufactured in each of two trials. Cheese 1, the test cheese, was manufactured using the bacteriocin (lacticin 3147) producing starter culture *Lactococcus lactis* DPC 3147. Cheese 2, the control cheese was manufactured using *Lactococcus lactis* DPC 5399 which was identical in all respects to DPC 3147 except it was unable to produce the bacteriocin - lacticin 3147. After curd formation the cheese was brined and smeared with a commercial preparation of smear bacteria comprising of *Debaryomyces hansenii, Staphylococcus xylosus*, *Arthrobacter globiformis, Brevibacterium lines* and *Geotrichum candidum*. Cheeses were washed at regular intervals to ensure even distribution of the smear and ripened at 16°C for two weeks followed by two weeks at 8°C. Samples were taken on the day of manufacture, post brining and smearing and subsequently at weekly intervals for 28 days. Survival of MAP was monitored weekly for the 28 days of ripening by plating on selective media. The presence of the bacteriocin was monitored during manufacture and ripening. The chemical composition of the cheese was also determined to ensure conformation to the norms for smear ripened cheese in terms of salt, pH and moisture. DNA was extracted from the cheeses at each time point and analysed quantitatively

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for the presence of MAP DNA using Real Time-PCR (RT-PCR).

4. Main results:

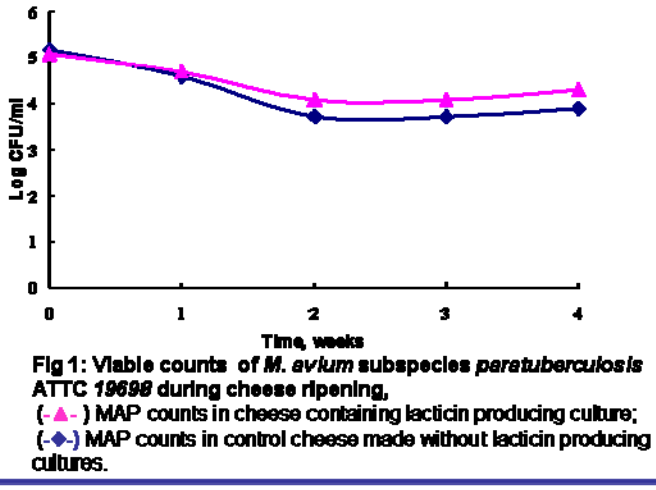

Fig 1: Viable counts of *M. avium* subspecies *paratuberculosis* ATTC *19698* during cheese ripening,
(-▲-) MAP counts in cheese containing lacticin producing culture;
(-◆-) MAP counts in control cheese made without lacticin producing cultures.

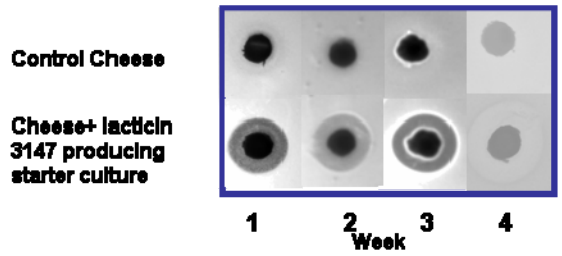

Fig 2: Well diffusion assays showing that lacticin 3147 could be detected in the cheese matrix throughout ripening

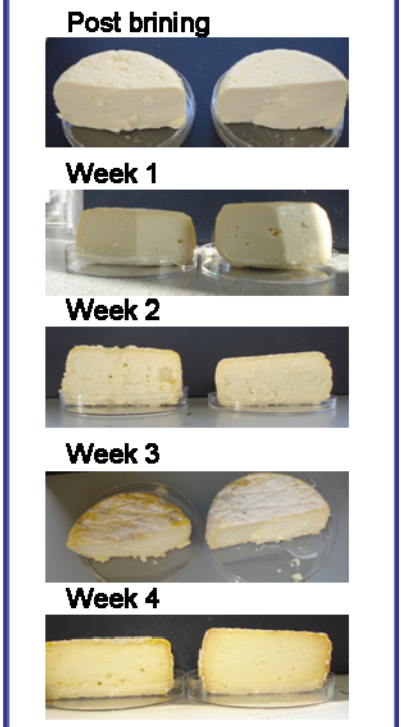

Fig 3 Raw milk smear cheese showing the development of the smear during the four weeks of ripening

1. Plating on selective agar indicated that viable counts of *Mycobacterium avium paratuberculosis* decreased less than 10 fold after 4 weeks of ripening. The molecular method, RT-PCR confirmed these results (Fig 1).
2. The presence of lacticin 3147 in the cheese matrix did not impact on the survival of MAP in the test cheese
3. Lacticin 3147 was detected in the test cheese throughout the manufacture and ripening of the cheese (Fig 2).
4. The use of lacticin producing starter cultures did not impact on the ripening of the cheese. Cheese on left is the control cheese, cheese on the right is made with lacticin 3147 producing starter
5. (Fig 3)
6. Because of the high microbial load associated with smear ripened cheese, the recovery of MAP by culture methods required the contamination of the cheese milk with MAP cells at levels exceeding those normally associated with naturally contaminated milk (which would normally not exceed 100/ml).
7. RT-PCR may prove a valuable tool for the future assessment of the survival of MAP in raw milk smear type cheese, owing to the increased sensitivity of this technique over traditional plating methodologies. Therefore, MAP counts closer to those normally found in naturally contaminated milk could be used in future studies.
8. Bacteriocin producing cultures may demonstrate greater efficacy when levels of MAP are in the normal range for naturally contaminated milk.

5. Opportunity/Benefit:

Results suggest that MAP would survive the manufacture and ripening of a raw milk smear cheese. However as the inoculum level used was greater than that likely to be found in naturally contaminated milk molecular methods such as RT-PCR would provide a more sensitive method than conventional plating methods for

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detection of low numbers of MAP present in an environment with a high microbial load such as that pertaining in a raw milk smear type cheese. MAP is more likely to be present in low numbers in naturally contaminated milk. However raw milk from animals shedding high numbers of MAP cells should not be used for the manufacture of smear type cheese without first undergoing pasteurization.

6. Dissemination:

Annual EU progress reports
Presentation at annual meeting of ParaTB Tools project Leon July 2009
Presentation to ParaTB Tools conference, Inverness, Scotland, 26-28th May 2010
http://www.vigilanciasanitaria.es/paratbtools/partners.php

7. Compiled by: Dr. Mary Rea, Dr. Tom Beresford, Dr. Sheila Morgan, Prof. Paul Ross

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