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Mycobacterium avium subsp. *paratuberculosis**

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Introduction

Over 100 years ago German scientists, Johne and Frothingham, isolated acid-fast bacilli from ruminant animals with a wasting disease characterised by chronic granulomatous inflammation that principally affected the terminal ileum (8). The acid-fast bacterium isolated was subsequently named *Mycobacterium enteritidis chronicae pseudotuberculosae bovis johne* and the animal disease became known as Johne's disease or Paratuberculosis. The current nomenclature for the causative agent of Johne's disease is *Mycobacterium avium* subsp. *paratuberculosis* or MAP.

Johne's disease is not currently classified as a zoonotic disease. However, there is a human disease called Crohn's disease that resembles Johne's disease in several respects and so a possible association between MAP and Crohn's disease in humans has long been suspected. This paper provides information on the organism and its veterinary and potential public health significance, presents evidence of its occurrence in animal-derived food and also in water, and outlines the current position in relation to MAP as an emerging human pathogen.

MAP – the organism and its detection

MAP belongs to the family Mycobacteriaceae, which also includes the tuberculosis and leprosy-causing species *Mycobacterium tuberculosis* and *Mycobacterium leprae,* respectively. In common with other mycobacteria its cell wall contains mycolic acids and it is acid-fast, resisting decolourization with acidified alcohol because of the presence of this waxy material. Under the microscope MAP cells appear as plump rods 1–2 µm in length which typically occur as clumps of up to several hundred bacterial cells. MAP is the slowest growing of the cultivable mycobacteria and primary culture from veterinary/clinical or food specimens can take 3–4 months or longer. The organism has a requirement for the incorporation of the iron-chelating compound mycobactin J into any complex medium used for its

*Lecture presented at the 38th Symposium of the Swiss Society of Food Hygiene, Zurich, 16. September 2005 cultivation. This mycobactin dependency represents a characteristic unique to MAP. Despite its inadequacies (slowness, lack of a selective culture medium, need to chemically treat sample to inactivate non-acid-fast microorganisms) culture remains the only means of demonstrating the presence of viable MAP in a sample (7). Until recently, the insertion element IS900 was thought to be a unique identifier for MAP and this formed the basis of molecular detection methods for MAP, such as PCR assays. Novel and more specific DNA target sequences for MAP are now needed and these will no doubt be identified as a result of the recent publication of the sequence of the complete genome of MAP (10). MAP is an obligate pathogenic parasite of animals, so the only place it can multiply in nature is in a susceptible host, within a macrophage. When MAP leaves an animal, for example in faeces or milk, it can survive for extended periods in soil and water, but it is unable to multiply outside the host. There is evidence that MAP can exist in vegetative, cell wall-deficient (sphaeroplast) and dormant forms.

Veterinary significance of MAP

MAP is the causative agent of Johne's disease (or paratuberculosis), a chronic, infectious enteritis which can affect a variety of animals, most commonly domestic ruminants such as cattle, sheep, and goats (2, 8). MAP can also infect other animals, such as red deer, rabbits and other non-ruminant wildlife species. The Office International des Epizooties (OIE) considers Johne's disease a disease of major global importance and it categorises Johne's disease as a List B transmissible disease, considered to be of socio-economic and/or public health importance within countries and significant in the trade of animals and animal products. Johne's disease has been reported on every continent and its prevalence is increasing in many countries, particularly in dairy cattle. When an animal is exhibiting clinical symptoms of Johne's disease (persistent diarrhoea, weight loss, progressive emaciation), and comes to the attention of the farmer, it has reached the clinical stage of the disease. It has been estimated that for every clinical case of Johne's disease existing in an infected herd as many as 4–8 other animals may have subclinical disease and be asymptomatic carriers of infection (11). These animals are infected by MAP but are not yet exhibiting the characteristic signs of Johne's disease. However, they are shedding the organism periodically in both faeces and milk. The main problem with Johne's disease in many respects is that it represents a hidden threat for farmers. Although most animals are infected at an early age, the onset of clinical symptoms is usually delayed by several years (typically 2–5 years). In the intervening years between infection and clinical manifestation of Johne's disease, asymptomatic carrier animals will have been contaminating the farm environment, potentially infecting other animals in the herd, or will have been sold on to another farmer thereby spreading the disease to other herds.

Potential public health significance of MAP

A possible association between MAP and Crohn's disease in humans has long been suspected but is currently not proven (1, 6, 10). Crohn's disease is a chronic inflammatory bowel disease of unknown aetiology that typically affects young people in the 15–25 year age group. The clinical and pathological resemblance of Crohn's disease and Johne's disease was first recognised as long ago as 1913. The two diseases share common symptoms (chronic weight loss and diarrhoea) and common pathology (ileum most frequently involved and granulomatous inflammation), but there are also distinct differences. Opinion in the gastroenterology community remains divided about the role, if any, of MAP in Crohn's disease (1). The current thinking regarding the aetiology of Crohn's disease is that it is multifactorial with genetic predisposition (Nod2 mutations on chromosome 16), exogenous factors (perhaps an infectious agent such as MAP) and host factors (e.g. "leaky gut", vascular supply, hormones, neuronal activity) acting together to induce a chronic state of dysregulated mucosal immune function (1).

Several expert groups have reviewed the available scientific evidence (cultural, PCR and serological) suggesting a link between MAP and Crohn's disease over recent years (reviewed by Grant (6)). The consensus opinion of all of these reviews is that the definitive evidence proving a causal relationship between MAP and Crohn's disease is not available. There is certainly evidence of an association (the occurrence at the same time and in the same patient of MAP and Crohn's disease) but not necessarily of causation (the organism has directly initiated the disease in the patient). The most recent review concluded that if MAP is involved in Crohn's disease it is not acting as a conventional infectious agent.

MAP in food and water

If MAP does have a role in Crohn's disease in humans then the organism may be transferred to humans via animal-derived foods or water. The most likely candidates as vehicles of transmission of MAP from animals to humans are milk (and potentially other dairy products), beef from older cows and water (5, 6). Cows' milk has received most attention to date because the ingestion of MAP-infected colostrum or milk is recognised as a primary means of transmission of Johne's disease from cow to calf, and to control the spread of Johne's disease within a herd it is recommended that calves are not fed such milk. Cows' milk forms a significant element of the diet of most people, particularly that of young children, and therefore transmission of MAP infection to vulnerable groups via milk is plausible. As most milk is consumed after pasteurization the focus of much of the research on MAP in milk has been to assess the efficacy of milk pasteurisation to inactivate MAP.

Numerous pasteurisation studies have been carried out over the past decade or so (summarized in a recent review by Grant (6)). Survival of low numbers of MAP after pasteurisation has been observed in the majority of studies involving both artificially spiked (11 of 14 studies) and naturally infected (6 of 9 studies) milk, to date.

In this author's opinion, factors such as the volume of milk tested, the application of chemical decontamination before culture, and the time of testing (immediately after heating or after refrigerated storage) probably led to non-recovery of low numbers of viable MAP from pasteurised milk in some of the other studies. Several studies involving naturally infected milk and commercial pasteurisers now report the presence of viable MAP in pasteurised milk and it is becoming more widely accepted that MAP may survive HTST pasteurisation on occasion. Estimates of the number of MAP surviving HTST pasteurisation are low (4–20 CFU/50 ml, 10–20 CFU/150 ml, and 0.002–0.004 CFU/ml). The public health consequence of low levels of MAP in pasteurised cows' milk being periodically consumed by susceptible individuals remains uncertain.

The mechanism that enables MAP to survive pasteurisation remains to be fully elucidated. Protection of cells within clumps from the lethal effects of heat is widely discounted since heat penetration into clumps should be instantaneous. However, given that greater inactivation of MAP cells was reported when clumps were disrupted by homogenisation prior to pasteurisation, clumping appears to have some influence on survival. A further hypothesis put forward to explain the presence of low numbers of *MAP* in pasteurised milk is heat activation of MAP cells above certain temperatures and extended holding times.

Johne's disease can also affect sheep and goats so MAP may contaminate the milk of these domesticated animals. MAP DNA has been detected in raw goats' milk in the UK (1.1 % positive), Norway (7.1 % positive) and Switzerland (23.0 % positive) by IS900 PCR, but either culture was not attempted or the presence of viable MAP was not confirmed in any of these studies. Raw sheep's milk was also tested. In a UK study there was no cultural or PCR evidence of MAP in sheep's milk, whereas in the Swiss study 23.8 % of raw sheep's milk samples tested MAP positive by PCR.

The first reports of viable MAP being isolated from retail cheese and powdered infant formula were published recently. *Gazouli et al.* (4) tested 42 samples of retail Feta cheese (made from a mixture of sheep and goats' milk) from Greece and 42 samples of hard, semi-hard and soft cheese from the Czech Republic, and reported the detection of MAP by IS900 PCR in 50 % and 12 % of samples, respectively, and the isolation of viable MAP from three cheese samples overall (3.6 %). *Hruska et al.* (9) tested 51 samples of powdered infant formula marketed by 10 manufacturers from seven European countries and detected MAP DNA in 49 % (n=25) samples and cultivated viable MAP from one sample. More extensive surveillance of these and other dairy products is warranted in order to establish potential risk posed to consumers.

In the advanced stages of Johne's disease in cattle, MAP infection is widely disseminated throughout the animal including muscle, lymph nodes and blood. It has, therefore, been suggested that meat from old dairy cows, used to make ground beef for human consumption in some parts of the world, may represent a source of MAP infection for consumers. It is hypothesised that when ground (minced) beef is prepared, localised infection (for example in a lymph node) could be spread throughout a whole batch of ground beef. To date, there is no scientific evidence to substantiate this theoretical risk of MAP in beef. The only reported survey of 113 minced beef samples collected from a single meat processing plant in the Republic of Ireland over a 4-month period found no evidence of viable MAP (see Grant (6)). It should be noted, however, that details of the culture methodology adopted in this survey are not published and it is possible that a sub-optimal recovery method was employed for testing the beef. More extensive surveillance is needed to determine if beef is a significant potential vehicle of transmission of MAP to humans.

Water may also represent a potential vehicle of transmission of MAP to humans (see Grant (6)). Surface waters contaminated by run-off from pastures grazed by animals with Johne's disease, and therefore potentially infected with MAP, could theoretically enter domestic water supplies. Everyone drinks water in its natural state or as a constituent of other beverages, and water is also used in food manufacture and processing so may reach consumers in that way. A survey of untreated surface water entering nine water treatment works throughout Northern Ireland, chosen to give a geographical spread and to be representative of different types of geophysical catchment areas, was carried out over a 12-month period. MAP was detected by IMS-PCR, culture or both methods in 15 (7.8 %) of 192 1-litre water samples tested. Water entering eight of the nine water treatment works showed signs of MAP infection at least once during the survey. Treated water was not tested during the survey and so the efficacy of water treatment to remove or inactivate MAP has not been verified. So far, only the effect of chlorination on inactivation of MAP in spiked water has been assessed. Various free chlorine concentrations (0.5, 1.0 and 2.0 µg/ml) and contact times (15 and 30 min), typical of those used for domestic water treatment, were studied. Results indicated that treatment with 2.0 µg/ml free chlorine for 30 min achieved the greatest inactivation of MAP in spiked water (2.35–2.82 $\log_{10}$ reduction), but complete inactivation was not achieved by chlorine treatment alone when high numbers of MAP (10^6 CFU/ml) were initially present in water. Chlorination on its own is generally only applied to pristine upland waters and ground waters. It is more commonly applied in combination with other water treatment processes so there is an urgent need to investigate the ability of other water treatment processes to remove or inactivate MAP.

MAP – the current position

Article 7 of EC Regulation No 178/2002 describes the precautionary principle and states that: "In specific circumstances where following an assessment of available information, the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures to ensure the high level of health protection chosen in the Community may be adopted, pending further scientific information for a more comprehensive risk assessment" (3). As there remains a degree of uncertainty about the role of MAP in human disease but there is evidence of MAP being present in cows' milk, both raw and pasteurised, and other dairy products, a precautionary approach is warranted in order to minimise possible human exposure to MAP via food and water. The levels of MAP detectable in cows', sheep and goats' milk from various countries are a reflection of the prevalence of Johne's disease in the national herds, so action is needed to reduce the prevalence of Johne's disease, particularly in the dairy cattle population, worldwide. If MAP infection in the animal population can be reduced by control programmes and promotion of animal husbandry practices that reduce the spread of MAP infection within herds (12), the levels of MAP in animal-derived foods will decrease accordingly and the risk of human exposure to this potential pathogen will be reduced. However, it must be recognised that there is no quick fix for Johne's disease and any reduction of MAP in food will take time to achieve and will require a concerted effort by government agencies, farmers, farming organisations, veterinarians and the dairy industry.

Summary

Mycobacterium avium subsp. *paratuberculosis* (MAP), the cause of Johne's disease in cattle, sheep and goats, may have a role in Crohn's disease in humans. Animals with Johne's disease shed viable MAP in their milk and faeces. The organism is also widely disseminated in the blood and tissues of infected cattle. Consequently, transmission to humans via consumption of animal-derived foods is a distinct possibility. Milk, other dairy products, beef and water have been identified as possible vehicles of transmission of MAP to humans. To date, viable MAP has been cultured from raw cows' milk, raw sheep and goats' milk, retail pasteurised cows' milk, and some retail cheeses in several countries. MAP has not been isolated from retail beef to date, although limited testing has been carried out. The public health consequences, if any, of low numbers of viable MAP being periodically consumed by susceptible individuals are uncertain. An association between MAP and Crohn's disease is not proven, but neither can it be discounted on the basis of current evidence. A precautionary approach is, therefore, warranted in relation to the existence of MAP in food, and action is needed to reduce the prevalence of Johne's disease in the cattle population worldwide, in order to minimise public exposure to this potential human pathogen.

Zusammenfassung

Mycobacterium avium subsp. *paratuberculosis* (MAP), ein säurefestes, schwach grampositives, aerobes, unbewegliches Stäbchen gehört zum *M. avium* Komplex (MAC). MAP gelten als Erreger der Paratuberkulose oder auch Johne'schen Krankheit, einer weltweit verbreiteten, ansteckenden, chronisch verlaufenden Darmentzündung der Wiederkäuer. Erkrankte und subklinisch infizierte Tiere scheiden den Erreger sowohl in der Milch als auch in den Fäces aus. Der Erreger kann zudem auch aus Blut und Gewebe von an Paratuberkulose erkrankten Tieren isoliert werden. Milch, Milchprodukte, Fleisch und Wasser werden als mögliche Vektoren für

eine alimentäre Übertragung zum Menschen beschrieben. In verschiedenen Ländern wurden MAP kulturell aus Rohmilch, pasteurisierter Milch und einigen Käsearten isoliert. Ein möglicher kausaler Zusammenhang zwischen *M. avium* subsp. *paratuberculosis* und dem Morbus Crohn beim Menschen ist aufgrund der bestehenden Datenlage bis heute weder bewiesen noch widerlegt. Vor diesem Hintergrund ist eine präventive Strategie zur Reduzierung eines möglichen Eintrages von MAP in die Lebensmittelkette anzustreben.

Résumé

Mycobycterium avium subsp. *paratuberculosis* (MAP), la cause de la maladie de Johne chez les bovins, les moutons et les chèvres pourrait jouer un rôle dans la maladie de Crohn chez l'humain. Les animaux atteints de la maladie de Johne rejettent des MAP viables dans le lait et les fèces. Cet organisme est aussi largement disséminé dans le sang et les tissus des bêtes infectées. Par conséquent, une transmission à l'homme par la consommation d'aliments carnés est probable. Le lait, les autres produits laitiers ainsi que la viande de bœuf et l'eau ont été identifiées comme de possibles vecteurs de transmission de MAP à l'homme. A ce jour, des MAP viables ont été cultivées à partir de lait cru de vache, de brebis et de chèvre, de lait pasteurisé commercialisé, ainsi que de fromages et ceci dans plusieurs pays. Actuellement MAP n'a pas pu être isolé à partir de viande de bœuf commercialisée, bien qu'un nombre limité de tests aient été effectués. Les conséquences pour la santé publique, s'il y en a, de la consommation périodique d'un petit nombre de MAP viables par des personnes à risque sont incertaines. L'association entre la MAP et la maladie de Crohn n'est pas prouvée mais ne peut pas être réfutée sur la base des données actuelles. Une approche se basant sur la présence de MAP dans les aliments démontre que, à titre préventif, une action est nécessaire afin de diminuer la prévalence de la maladie de Johne dans les troupeaux de bétail à travers le monde, et par conséquent de diminuer l'exposition du public à ce germe potentiellement pathogène pour l'homme.

Key words

Mycobacterium avium subsp. *paratuberculosis,* Crohn's disease, Johne's disease, milk, pasteurisation, beef, water

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