Mycobacterium paratuberculosis
Does it contribute to Crohn's disease?

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Foreword

Crohn's disease is a condition in which the wall of the bowel of humans becomes inflamed and thickened. Sometimes inflammation may also occur in other parts of the body. Crohn's disease is a very variable and unpredictable condition. Some people affected by this disease have very severe problems with pain, inability to absorb food properly and diarrhoea almost continually unless they have treatment. Others have very long periods of relatively good health with occasional episodes of "flare up" of the disease.

The cause of Crohn's disease is not known. There is strong evidence to support the belief that the immune system of those with Crohn's disease behaves differently from that of most people and that this is part of the reason for the inflammation in the wall of the bowel. There are also reasons to believe that items in the diet or the environment may contribute to the inflammation. One of the possible contributory factors that has received attention in recent years is a species of bacteria called *Mycobacterium paratuberculosis.* This bacterium has been known for many years as a cause of Johne's disease, a bowel disease in cattle. Johne's disease is uncommon in Ireland. Johne's disease in cattle has a number of similarities with human Crohn's disease but they are not identical diseases. It is known that cows infected with *M. paratuberculosis* do not appear ill for several years and so can continue to provide milk for human consumption. It is also known that these bacteria can enter the milk of infected animals. Recent evidence also suggests that some strains of *M. paratuberculosis* may be less easily killed by pasteurisation than many other kinds of bacteria.

In this report we have sought to evaluate the available, sometimes conflicting, evidence on the possibility that *M. paratuberculosis* represents a risk to human health. Many questions that might be readily addressed in respect of other kinds of bacteria are much more difficult to answer for this species as the organism is difficult to grow in the laboratory. Given the existing state of knowledge we have recommended that such measures as are reasonably practical should be taken to minimise exposure of the public to this organism. These measured recommendations reflect a consciousness that the world we live in and the food we eat is not free from bacteria and that efforts to render it so may be counterproductive, in terms of the quality and variety of the food we eat. Even as this report is published new research will no doubt appear in relation to *M*. *paratuberculosis* and Crohn's disease and it will be necessary to keep this topic under review in the light of what will be learned over the coming years.

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What is Crohn's disease?

Crohn's disease is a chronic inflammatory disease of which the cause or causes are not established. [5,10,13,14,16,22,27]. Although primarily considered a disease of the small intestine it can affect any part of the gastro-intestinal tract and sometimes extra-intestinal body sites. Current concepts regarding the cause of Crohn's disease emphasise a dysfunction of the immune system resulting in a prolonged and intense process of inflammation. The damage to the bowel appears to be due to this inflammatory process [5,12,22,27,31]. It is generally accepted that people who develop Crohn's disease have some inherited characteristic which makes them susceptible to the development of Crohn's disease but also that some environmental factor or factors[15] are required to initiate the disease, in addition to the inherited characteristic[5,13,14,16]. An immune response resulting in inflammation may be triggered by a living bacterium or virus or by non-living substances such as fragments of dead bacteria, elements of food etc. Environmental factors might be important not only in starting the process of inflammation but also in maintaining the process. It is possible that, even in a particular person with Crohn's disease, any one of several environmental factors may contribute to triggering or maintaining the disease [13,14]. Theories in relation to environmental factors include microbes of several types including *Mycobacterium paratuberculosis* [3,7,8,11,15,21,28,29,30,32,33] and other microbes normally present in the large intestine [1,23,35]. In addition, substances in food, toothpaste and exposure to measles and measles vaccine have been or are being investigated as possible trigger factors[6,10,18,25].

What is *Mycobacterium paratuberculosis*?

The genus *Mycobacterium* is a large group (more than 70 species) of bacteria[24]. Although the best known species are *M. tuberculosis* and *M. bovis,* which are associated with human and bovine tuberculosis respectively, other species may cause disease in animals or exist in the environment and rarely or never cause infection in otherwise healthy humans. *Mycobacterium paratuberculosis* is the causative agent of Johne's disease in cattle[4,9]. It is difficult to study because it grows very slowly and only under very specific conditions in the laboratory [9]. Many recent studies of *M. paratuberculosis* have used detection of DNA using the Polymerase Chain Reaction (PCR) because of the difficulty in growing this organism [3,7,11,15,21,28,32].

M. paratuberculosis is differentiated from the great many mycobacterial species which are common in the environment by its clearly established ability to cause bowel disease in cattle and other animal species. It is not known at the present time if *M. paratuberculosis* is capable of causing bowel disease in humans but in rare cases it may be associated with infection of the lymph nodes [3,9,30].

Is there a link between *Mycobacterium paratuberculosis* and Crohn's disease?

The pathological changes in the small intestines of Crohn's disease sufferers can closely resemble those found in cases of Johne's disease in cattle[11].

M. paratuberculosis has been cultured from the intestinal tract of some individuals with Crohn's disease (overall about 7.5% of those studied)[3]. The organism has also been cultured from healthy individuals but much less frequently (less than 1% overall)[3]. Some of the evidence implicating *M. paratuberculosis* in Crohn's disease is based on the detection of *M. paratuberculosis* nucleic acids using PCR[3]. Although PCR is capable of detecting very low levels of *M. paratuberculosis* nucleic acids, in most studies the methods used are not capable of determining if the nucleic acid amplified originated from living or dead bacteria.

Summary: Current evidence suggests that although *M. paratuberculosis* bacteria and *M. paratuberculosis* DNA are not detectable in most people with Crohn's disease, they may be detectable more commonly in the gastrointestinal tract of those with Crohn's disease than in individuals without Crohn's disease[3].

Is *Mycobacterium paratuberculosis* a cause of Crohn's disease?

The inconsistent results of studies designed to demonstrate the presence of *M. paratuberculosis* in the bowel of those with Crohn's disease, when considered together with the evidence for the importance of other hereditary and environmental factors, suggest that if *M. paratuberculosis* is involved in the etiology of Crohn's disease it is probably not the sole cause[3,13,14].

Although beneficial effects of treatment with "anti-mycobacterial" therapy have been reported[19], the antibiotics used in most cases are also active against many other species of bacteria[19,20]. Beneficial effects of treatment have also been reported with antibiotics which are not known to have any useful effect on mycobacterial infection[13].

It should be noted that immuno-supressant drugs rather than anti-bacterial treatment is the standard approach to the treatment of Crohn's disease[12,13] and that although AIDS is associated with increased susceptibility to infection with many species of mycobacteria, individuals with Crohn's disease and AIDS may experience remissions of Crohn's disease[34]. These factors suggest that if bacteria or substances of bacterial origin are involved in Crohn's disease it is possible that a dysfunctional immune response to these factors may be as important or more important than the bacteria themselves.

Is milk a potential or actual hazard in the cause or exacerbation of Crohn's disease?

The current incidence of Johne's disease in Irish cattle is low. In 1997 the Department of Agriculture, Food and Rural Development reported 12 diagnosed cases. The condition is notifiable. It is conceivable that a certain amount of under-reporting may occur since there is little obvious financial advantage to the farmer in reporting cases.

Large numbers of cattle intended for breeding and milk production are imported each year from other Member States of the European Union. Intra-community law does not require a test for Johne's disease as a condition of trade. It must be said that the diagnostic tests currently available have a poor reputation for both sensitivity and specificity except in animals already showing clinical signs[4].

It has been shown that milk from cows affected with Johne's disease may carry *M. paratuberculosis*[9]. This appears to be most likely before or during the period in which clinical signs are present.

The sale of unpasteurised cows' milk for direct human consumption has been banned since 1 August 1997. The preparation of cheese from raw milk is permitted under certain conditions but these conditions do not make specific reference to Johne's disease or testing for Johne's disease.

In respect of unpasteurised milk it is important to note the following:

a) Crohn's disease is more common in developed than in undeveloped countries[27]. Some of the differences in rates of diagnosis may be due to differences in the level of health care provided,
b) Crohn's disease may be more common in urban than in rural areas[3], whereas the level of consumption of unpasteurised milk (and incidentally of contact with animals suffering from Johne's disease) is considerably higher in rural areas,
c) since dead bacteria or substances derived from bacteria may contribute to initiating or sustaining an immune response, it is not possible to be certain that inactivation of any bacteria which might contribute to an inflammatory process in the gastrointestinal tract would render the bacteria immunologically inert,
d) there is evidence that the time/temperature combination established in European Community and national law as the minimum required for effective pasteurisation of milk (71.7°C for 15 seconds) does not render all *M. paratuberculosis* non-viable, however the experimental work done in this area has been based almost entirely on seeded samples pasteurised under laboratory conditions [2,11].

There is a body of opinion which holds that, while the incomplete inactivation of the bacteria by pasteurisation, under laboratory conditions, results from

clumping, which effectively protects at least some of the organisms from the full effects of the heat applied, the relative turbulence of the flow of milk through commercial pasteurisers prevents clumping and thus allows for a higher "kill" [26]. This, coupled with the much lower burden of bacteria resulting from the dilution effect of bulking milk from a large number of cows, is one of the bases for an optimistic view of the risk involved. On the other hand, one study indicates that samples of milk collected in the London area over a period of two years gave a positive result for *M. paratuberculosis* in 7% of cases when examined by the IS900-PCR method [17]. As previously stated, this PCR approach does not differentiate between viable and non- viable organisms. Attempts to culture the organism from the milk samples in this study were unsuccessful.

Is there a pasteurisation procedure which will inactivate all *Mycobacterium paratuberculosis* without causing unacceptable changes to the taste of milk?

Some research conducted under laboratory conditions suggests that, in order to ensure inactivation of all *M. paratuberculosis* both the temperature and the length of time of pasteurisation may need to be raised from the present accepted levels[11]. The research suggests that time is more important than temperature, but no firm guidelines as to the most effective level of either, under commercial conditions, have emerged.

In practice the dairy industry pasteurises at a temperature two or three degrees above the legal minimum of 71.7°C for 15 seconds to make certain that there is no risk of falling below this level of treatment. However, there is an upper temperature beyond which unacceptable changes to the taste of milk start to occur.

Any increase in the temperature and/or time of commercial pasteurisation involves additional cost in generating and applying the extra heat, although of course this is not a consideration where such measures are clearly warranted for in the protection of public health.

Is there a method of ensuring that a modified pasteurisation process is properly applied in practice?

If a temperature/time combination which inactivates all *M. paratuberculosis* is determined and if implementation of such a process was considered justified, then a modification of, or substitute for, the phosphatase test would be needed which would indicate, accurately and rapidly, that effective treatment has been achieved. This would call for development work both in the laboratory and, more importantly, under commercial conditions.

Are specific measures for control of *Mycobacterium paratuberculosis* required in cattle?

M. paratuberculosis is an important animal pathogen and has the potential to cause problems involving animal welfare as well as economic losses in the dairy industry. On this basis, efforts to control the spread of the disease among animals appear appropriate. If *M. paratuberculosis* is harmful to human health, such efforts would also contribute to the protection of public health. Johne's disease is a scheduled and notifiable disease under Irish veterinary legislation. Diseased animals are removed. No human health related measures are taken in relation to the remainder of the herd.

It is accepted that the clinical presentation of Johne's disease occurs very late in the course of infection. The tests available for the detection of *M. paratuberculosis* infection in individual animals prior to development of clinical disease are not highly sensitive**.** Because of the limitations of existing diagnostic tests it is difficult to identify additional infected but healthy animals in a herd in which a clinical case of Johne's disease has been detected.

Mycobacterium paratuberculosis infection in humans

If *M. paratuberculosis* does cause or contribute to Crohn's disease in humans, the mechanisms by which it may do so are uncertain. It is not clear to what extent viable *M. paratuberculosis* survive commercial pasteurisation. It is conceivable that if *M. paratuberculosis* contributes to a chronic inflammatory process in the human gastrointestinal tract, that it may do so even if rendered non-viable, since it would remain immunogenic even if non-viable.

Effective research is rendered difficult for a number of reasons:

(i) by the nature of the organism, as it is difficult and slow to grow in the laboratory

(ii) by the nature of the disease caused in bovines, due to the long incubation period

(iii) by the limitations of existing technology for detection of infection in the sub- clinical phase of bovine infection, and

(iv) by the low incidence of Johne's disease in cattle in this country.

Measures to prevent human infection with *Mycobacterium paratuberculosis* through the consumption of milk and milk products - current position

No specific precautions are currently in place in respect of *M. paratuberculosis* in milk in any other country, even in those with a high incidence of Johne's disease in dairy cattle. Any change in pasteurisation procedures would require legislative changes and, in the context of the European Union and the single market in milk and milk products, these would have to be Community-wide rather than simply national.

Briefly, the decisions to be made are as follows:

1. should measures be taken to control human exposure to this known animal pathogen if there is uncertainty as to whether or not the organism can cause harm to human health?
2. what measures are practical and justifiable in the current state of knowledge?

Issues for consideration

1. Given that the incidence of Johne's disease is low in Ireland and that diagnostic tests are not very reliable, testing herds may not be practical or necessary.
2. A small number of clinical cases of Johne's disease in animals will arise and it is possible that pre-clinical infection exists in some other members of at least a proportion of such herds. It is very likely that *M*. *paratuberculosis* will be present in the milk derived from *Mycobacterium paratuberculosis* infected animals for a period prior to the development of clinical disease. In principle it may be possible to reduce the level of exposure of the human population to viable *M. paratuberculosis* by requiring that milk from dairy herds in Ireland, in which a clinical case of Johne's disease has been diagnosed, be pasteurised before use for human consumption. In practice all milk sold for human consumption from dairy herds in Ireland is already pasteurised, with the exception of a small fraction used for preparation of farmhouse cheese. It should also be noted that milk and milk products, including cheese produced from unpasteurised milk are imported into Ireland from countries in which no such controls are in place.
3. At present the only confirmed association between *M. paratuberculosis* and human disease is isolated case reports of infection of lymph nodes in the neck. In Europe infection of lymph nodes of the neck is far more commonly associated with *Mycobacterium avium*, an organism commonly found in water. On this basis it is not apparent that there is an imperative to prevent the possibility of low level exposure of the human population to *M. paratuberculosis,* which would justify the resource implications of additional testing, regulation or depopulation of herds.

4. Even if it is assumed that there is no evidence to suggest a link between *M. paratuberculosis* and Crohn's disease, it is a general principle that diseased animals are not to be used as a source of food for human consumption.

Conclusion

The question put to the Sub-committee asked whether or not *Mycobacterium paratuberculosis* contributes to Crohn's disease. The answer is inconclusive for several reasons:

Although we know that *M. paratuberculosis* causes Johne's disease in cattle and that the bacteria may pass into the human food chain via cows' milk, we do not know if the bacteria causes or contributes to Crohn's disease in humans, even though it has been reported that *M. paratuberculosis* has been detected more commonly in patients with Crohn's disease than in the general population (<1.0%).

- Diagnostic tests for Johne's disease are poor but without an extensive monitoring survey we cannot know the true incidence of the infection in dairy cattle. This makes it difficult to link the disease with the human Crohn's disease.
- Normal pasteurisation of milk at 71.7°C for 15 seconds kills low levels of *M. paratuberculosis*. However, experimental work in laboratory conditions has shown that at high levels of *M. paraturberculosis* normal pasteurisation does not render all of the bacteria non-viable. The effectiveness of increasing the time or temperature in the pasteurisation process has not been established and hence any potential benefit to human health cannot be determined.

There is a need to keep this subject under review as international research may provide clarification of the many areas of uncertainty over the coming years.

In the meantime the Sub-committee concludes that:

1. It is reasonable to prohibit the use of milk derived from animals with clinical Johne's disease in the interim between their identification and their removal from the herd. The use of milk from such animals for calves is likely to facilitate spread of infection and prohibition of its use for this purpose may also be reasonable.

2. Raw milk from farms where Johne's disease is current should not be used for human consumption or for use, unpasteurised, in the making of cheese.

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