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# *Mycobacterium avium* subspecies *paratuberculosis*: the nature of the problem

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*Mycobacterium avium* subspecies *paratuberculosis* (*MAP*), originally called Johne's bacillus, was first described from Germany in 1895 as the cause of a chronic inflammatory disease of the intestine in a cow. As the 20th century progressed, clinical and subclinical *MAP* infection in farm animals appeared to become more prevalent. Amongst the early reviews available are the excellent ones prepared by Doyle (1956) from the Veterinary Laboratory, Weybridge UK and Reimann and Abbas (1983) from the University of California, Davis. In general, the response on farms to the appearance of clinical Johne's disease was to cull infected animals. This practice over the course of the century may have exerted a selection pressure on *MAP* favouring the emergence of strains which can infect animals for years without necessarily causing clinical disease. In the latter part of the 20th century the incidence of clinical disease due to *MAP* in some areas of Western Europe and North America appeared to decrease. The problem which confronts us now is *subclinical MAP* infection of domestic livestock throughout Western Europe and North America, and the emergence of wildlife reservoirs including those in rabbits and their predators, carrion birds and badgers (Beard et al., 1999, 2001). In the United States and Canada the herd prevalence of *MAP* infection is reported in the range 21–54% (Collins et al., 1994; Johnson-Ifearulundu & Kaneene, 1999; Merkal, Whipple, Sacks, & Snyder, 1987; NcNab, Meek, Duncan, Martin, & Van Dreumel, 1991; Wells, Ott, Garber, & Bulaga, 1996). In Western Europe the herd prevalence lies in the same range, and a recent serological study of bulk-tanked milk from 900 dairy herds in Denmark reported that 47% of herds tested positive for *MAP* infection (Nielsen, Thamsborg, Houe, & Bitsch, 2000). What is beyond doubt is that *MAP* is widespread in our domestic animals.

Subclinically infected dairy cows may secrete *MAP* in their milk (Sweeney, Whitlock, & Rosenberger, 1992). It is one of the ways the organism passes from infected parent to offspring when the calf may be most susceptible. *MAP* is more robust than *M. bovis* or *M. tuberculosis* and the destruction of all viable *MAP* by exposure to current pasteurisation conditions of 72°C for 15 s is not assured. Research carried out in the Department of Agriculture and Rural Development for Northern Ireland, at Queen's University, Belfast, and reported by the Food Standards Agency (2000), found that small slow-growing, mycobactin-dependent, IS900 PCR positive colonies of *MAP* could be cultured from 1.7% of samples of retail pasteurised cows' milk widely obtained throughout Britain. *MAP*-positive samples included some exposed to the longer holding time of 25 s during pasteurisation. It is clear that the human population in the UK is being exposed to *MAP* in retail milk supplies. Other dairy products, as well as raw and processed meats, are also at risk. *MAP* accumulates particularly in the ileocolonic regions of the intestine where these organisms may remain for years and not cause clinical disease. This situation is similar in principal to the widespread exposure of human populations in Europe, North America and elsewhere, to *M. bovis* before the introduction of milk pasteurisation and the tuberculin testing of dairy herds in the middle third of the 20th century. With *MAP*, only those individuals with a particular inherited or acquired susceptibility may go on eventually to develop clinical disease.

Infected animals excrete *MAP* onto pastures. Wildlife reservoirs contribute to environmental contamination. The problems now being caused by *MAP*, differ from those previously caused by *M. bovis*, in that *MAP* can survive for long periods in the environment. Rains falling on contaminated land will wash *MAP* into

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surface waters and rivers. Although much research needs to be carried out in this area, it is probable that *MAP* in the environment is taken up into organisms such as amoebae in which they can survive. This may allow them to replicate, to increase their resistance to biocides and potentially acquire a phenotype which is more pathogenic for humans. Water abstracted from rivers and lakes contaminated with *MAP* may convey these organisms to human populations. *MAP* arriving at domestic outlets in high dilution may accumulate in biofilms lining household water systems. Either in the food chain therefore, or in water supplies, it is inevitable that humans sharing the same geographic areas with animals which are extensively infected, will be exposed to these pathogens.

The question as to whether *MAP* may also cause disease in humans has its origins in a proposition first published in 1913 (Dalziel, 1913). We have recently prepared a detailed analysis of this complex issue which is in general poorly understood, even by medical specialists in the field of chronic inflammatory diseases of the intestine (Hermon-Taylor et al., 2000). It is now known that *MAP* can cause chronic inflammation of the intestine in a very broad range of animals including large and small ruminants, monogastrics such as dogs and pigs, and so far, at least four types of sub-human primates. *MAP* shows a well-defined tissue tropism and will end up causing chronic inflammation of the intestine even if administered experimentally by subcutaneous, intravenous or intraperitoneal routes. The histopathological features of *MAP* disease in animals ranges from one in which millions of typical ZN-positive *MAP* are visible microscopically in the inflamed intestine to the other extreme where no *MAP* can be seen at all, but there is chronic granulomatous inflammation. This is just what leprosy, another mycobacterial disease, does in humans. One of the properties of *MAP* which has retarded our understanding of the problems it is causing that it may be very difficult to culture in the laboratory. Patient work in many laboratories has, however, shown that *MAP* can be grown in conventional culture from about 5% of people with Crohn's disease, but not from normal people. Cultures have had to be incubated for months or years before any growth identifiable by conventional means, becomes visible.

*MAP* is very similar to other organisms of the *M. avium* complex (MAC) to which we are all exposed, so that immunological tests for *MAP* infection in humans using crude extracts of laboratory cultured organisms usually report no difference between Crohn's disease and normal people. Recent studies have however shown that if specific targets on *MAP* are carefully selected, highly significant differences in immune recognition can be demonstrated between Crohn's disease and normal people (Naser et al., 2000a; Olsen et al., 2001). An important example of this has come from recent research at UCLA which showed that the blood of nine out of ten people with Crohn's disease contained IgA which recognised a mycobacterial protein richly expressed on *MAP* called HupB (Cohavy et al., 1990). HupB is identical to the laminin receptor used by *M. leprae* to enter Schwann cells round nerves causing the neural inflammation so characteristic of leprosy (Shimoji, Ng, Matsumra, Fischetti, & Rambukkana, 1999). Neural inflammation is long known to be specific feature in the inflamed gut in Crohn's disease (Geboes et al., 1992) and antibodies to the chronic inflammatory disease-associated autoantigen pANCA, cross-react with HupB.

In 1990, after spending 20 months carefully optimising sample processing and experimental procedures, we began a study which revealed the presence of *MAP* DNA in about two-thirds of people with Crohn's disease using IS900 PCR (Sanderson, Moss, Tizard, & Hermon-Taylor, 1992). It was also present in the intestine of 12% of normal people, which is just what would be expected to occur in a totally exposed population. Since then there have been 18 peer reviewed publications using a variety of experimental methods, nine of which reported the presence of *MAP* in CD gut some or most of the time, and nine found *MAP* hardly ever or not at all (Hermon-Taylor et al., 2000). Similar inconsistencies have occurred in the results of DNA tests applied to other chronic inflammatory diseases such as TB. Apart from some obvious methodological errors, the reasons for the uncertainty are the low abundance of *MAP* in Crohn's disease intestine and its extraordinarily tough protease-resistant phenotype. Recent research by Dr. Saleh Naser and colleagues from the University of Central Florida, using improved liquid cultures and IS900 PCR on their centrifugal pellets, has demonstrated *MAP* in 86% of surgically resected Crohn's disease gut (Schwartz et al., 2000). The same authors have also demonstrated *MAP* in the centrifugal pellets of breast milk from each of two mothers with Crohn's disease who had recently given birth, but not in the breast milk of five normal women (Naser et al., 2000b). Work in our own lab by Jun Cheng and Tim Bull, using much improved methods, found Chinese *MAP* in 69% of Chinese surgical path blocks from Crohn's disease patients in China, and in 14% of path blocks of normal intestine from Chinese people. The consumption of liquid cows' milk in China has increased substantially over the last 20 years. Taken together, these studies clearly demonstrate the presence of the chronic enteric pathogen *MAP* in a substantial majority proportion of humans with chronic inflammation of the intestine of the Crohn's disease type.

It has long been known that infections due to non-tuberculous mycobacteria in immunocompetant people, particularly those caused by MAC, are usually resistant to standard anti-tuberculous drugs. These organisms can prevent the drugs penetrating the microbial cell and

can rapidly develop mutations which confer drug resistance. Lasting resolution of *MAP* infections in animals using standard anti-mycobacterial treatment has never been convincingly demonstrated, and much the same outcomes have resulted from a similar treatment approach in humans with Crohn's disease (Hermon-Taylor et al., 2000). *MAP* are however more susceptible to some newer drugs which are man-made chemical modifications of natural streptomyces antibiotics, such as rifabutin and clarithromycin. These agents also have the advantage of being concentrated within macrophages where *MAP* in infected animals and in humans almost certainly resides. There are now four open clinical studies including our own initial work (Borody, Pearce, & Bampton, 1998; Douglass, Cann, & Bramble, 2000; Gui, Thomas, & Tizard, 1997; Shafran, Piromalli, & Naser, 2000) all of which say the same thing. This is that a substantial proportion of people with active Crohn's disease will go into remission with healing of the intestine which is sometimes lasting, when treated with combinations of these drugs. A randomised controlled trial of this treatment was initiated in Australia in September 1999.

A question which is frequently asked is …. 'how can so few *MAP* cause so much chronic inflammatory disease?' To answer this we must allow our thinking to escape from the immobilising presumption that it must be like TB, in which a major factor in the disease process is a direct immunological response to cell wall components. *MAP* in animals with the paucimicrobial form of the disease and in humans, does not have a classical mycobacterial cell wall. *MAP* colonising immunoregulatory cells like macrophages almost certainly causes an immune dysregulation. Together with defects in the integrity of the overlying mucosa, much of the disease itself is caused by an exaggerated immunological response to leakage into the gut wall of bacteria and food residues normally confined to the lumen. Clinical improvement can be achieved by suppressing or modulating the immune reaction, by reducing the allergic component and altering the enteric flora with elemental diets, and by treatment with antibiotics such as ciprofloxacin and metronidazole. Without killing the underlying causative pathogens however, the benefit which follows such treatments is rarely lasting.

Unlike mathematics and physics 'proof' may be difficult to achieve with biological problems. If the nature of the *MAP* problem outlined in this mini-analysis is correct, it suggests that we are challenged by a Public Health issue of substantial proportions for which a range of remedial measures is needed. We need to make sure our milk and water are safe, and that our animals are clean. We need to make Crohn's disease reportable so we have accurate data to monitor the effect of these measures on the overall problem. We need to make a rapid increase in the volume and intensity of research in the field. The availability of the total genome sequence of bovine isolate of *MAP*, currently nearing completion at the University Minnesota, will accelerate our ability to develop preventative vaccines for animals and therapeutic vaccines for *MAP*-infected humans with chronic inflammation of the intestine.

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