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IS *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* (*MAP*) A POTENTIAL FOOD-BORNE ZOONOTIC AGENT?

A. R. BURRIEL[1] , I. TSACHEV[2] & S. B. RAMANTANIS[3]

[1]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, University of Thessaly, Greece; [2]Department of Microbiology, Infectious & Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria; [3]Laboratory of Meat and Meat Products Technology and Quality Control, Department of Food Technology, Technological Educational Institution of Athens, Greece

Summary

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The spreading of *Mycobacterium avium (Map)* infections among animals is a representative example of an on-going evolution of microbes in the environment. However, is it or could it be a potential zoonotic agent? If it is proven as such, then how men are infected? What is the route of transmission? To this date there is only speculation on these questions. Crohn's disease in men resembles clinically and histopathologically Johne's disease in animals, and *Map* has been isolated from both, but it has yet to be proven as cause of clinical disease in humans. Regardless of the eventual outcome of on going research, food scientists should remember that the possible sources of infection of men with *Map* are feces from infected animals or men, containing large numbers of this microorganism, and food contaminated accidentally or by excretion from infected animals. Specifically, milk from infected animals is the most likely source, if pasteurization fails to kill the microorganism. Time, temperature of pasteurization and the number of microorganisms are critical for the effectiveness of pasteurization. Failing of pasteurization has been observed even with the high temperatures of ultra pasteurization, if bacterial numbers are high.

Key words: Crohn's disease, Johne's disease, *Mycobacterium avium,* paratuberculosis

INTRODUCTION AND BACKGROUND

The question "Is *Map* a zoonotic agent" has yet to be answered, although many scientists are currently working on an answer. To this date the possibility of *Map* being a zoonotic agent is still only speculation. *Map* infection of animals causes the clinical condition known as paratuberculosis or Johne's disease, which has a clinical and histological resemblance to Crohn's disease in men (Hermon-Taylor *et al*., 2000; Harris & Lammerding, 2001; Bull *et al.*, 2003). However, for *Map* inclusion among the zoonotic agents, one must prove this microorganism's involvement in the development of clinical disease in man. *Map* has been isolated

from the faeces of healthy individuals or those suffering from inflammatory bowel disorders, including Crohn's disease (Thompson, 1994). It has also been recently identified by PCR in intestinal tissue from Crohn's disease sufferers (Collins *et al.*, 2000; Bull *et al*., 2003). However, its presence is not enough to call it the causative agent of this disease. Reproduction of the clinical disease by experimental infection of man is impossible for ethical reasons, as well as the direct connection of *Map* infection and Crohn's disease. In addition, the interval between *Map* infection and appearance of clinical disease depends on time length, as well as on the method identifying the agent (Harris & Lammerding, 2001; Berstein *et al*., 2003; Bull *et al*., 2003). The time interval between infection and clinical disease observed in experimental and natural infections of animals is an example of why investigators of human infection are failing to firmly connect clinical disease and infection with *Map*.

The co-occurrence of this microorganism and Crohn's disease, a human wasting chronic condition, has increased speculation about the possibility of *Map* being implicated either as causative or secondary infecting agent in the development of Crohn's disease, thus being a zoonotic agent (Thompson, 1994; Chiodini & Rossiter, 1996; Shivananda *et al*., 1996; Mutwiri *et al*., 2001; Greenstein, 2003). This view is strengthened by the location of lesions to the ileum and the histopathological similarities of the clinical appearance of Crohn's disease to paratuberculosis in animals (Dunne *et al*., 1977; Chiodini *et al*., 1984; Cocito *et al*., 1994; Collins *et al*., 1994; Harris & Lammerding, 2001). However, *Map* has also been isolated or detected by PCR from healthy individuals, although among the latter with a lower prevalence than that of Crohn's sufferers (Thompson, 1994; Collins *et al*., 2000; Bernstein *et al*., 2003; Bull *et al*., 2003). Crohn's disease in men, like Johne's disease in animals, is a century old disease and continuously increasing in prevalence among industrialized people of the world. Regardless of overall prevalence, of concern is the age of the affected individuals (15-35 years of age). As a chronic illness of the ileum of young individuals, it has accumulative nature needing a life-long management and treatment (Harris & Lammerding, 2001; Bernstein *et al*., 2003; Vantini *et al.*, 2004). The way veterinarians manage suspect farms and animals will dramatically change should scientists prove that *Map* is a potential zoonotic agent. First measure should be intensification of detection of infected food producing animals aiming in helping veterinarians better understand the long term effects of animal infections on human health.

Infection of animals with *Map* varies between animal species, farm location and age of animals (Chiodini *et al*., 1984; Cocito *et al*., 1994; Collins *et al*., 1994). The observed variation is partly due to the peculiarities of this microorganism's pathogenicity, species infected or age, and partly to the problems faced by veterinarians in identifying animals suspect of infection. Culturing the microorganism from faeces is the only confirmation of infection. This, although confirmatory, is laborious, time consuming and possible only if it is attempted at times of shedding, thus positive for a small number of samples investigated. Growth of *Map* in culture media occurs after 18 weeks to years of incubation at 37^{o} C (Thore *et al*., 1990; Juste *et al*., 1991; Bull *et al*., 2003). Isolation is also very difficult if the number of colony forming units (cfu) of the microor-

ganism in the examined specimen is small. A positive result requires species identification, a process adding to the time needed for that identification, thus confirmation. Of course new methods, such as PCR, have being sought and are currently used experimentally with various degrees of success (Collins *et al*., 1989; McFadden *et al*., 1992; Millar *et al*., 1996; Ritacco *et al*., 1998; Bernstein *et al*., 2003; Bull *et al*., 2003). They need verification and the best way is by isolation of the microorganism (Collins *et al.,* 2000; Harris & Lammerding, 2001; Bull *et al*., 2003), which is an area of concern when scientists decide upon areas of research interests. In addition, PCR requires specialized laboratory and properly trained personnel to be either feasible or reliable. Thus, such methods are unpractical and expensive for routine use and epidemiological investigations of animal and man infections with *Map*. The easiest way of identifying animals infected with *Map* would be via indirect methods, such as serologic ones. Unfortunately, serologic investigations are largely ineffective in identifying infected animals. They are sensitive when clinical disease has developed, thus effective in testing older animals (Ridge *et al*., 1991; Billman-Jacobe *et al*., 1992; Perez *et al*., 1994; Perez *et al*., 1997; Harris & Lammerding, 2001).

Regardless of the eventual outcome of current speculation on the probable role of *Map* in Crohn's disease, discussing the possible routes of transmission to men is helpful in preparing for the most effective means of preventing human infection, thus consumers' panic. The present review is aiming in briefly examining the nature of paratuberculosis and Crohn's disease and discussing the possible routes of *Map* transmission from animals to men. In addition, areas where more research is needed are identified and suggestions are made in preventing human infection.

THE MICROBIAL AGENT

Map is a member of the Mycobacteriacea family. The family consists of slow growing species highly resistant to acids. *Map* is one of the many subspecies, potential or obligate pathogens, of *Mycobacterium avium* (Thore *et al*., 1990; Dumonceau *et al*., 1997; Hermon-Taylor *et al*., 2000; Harris & Lammerding, 2001; Bull *et al*., 2003). The species is divided to subspecies on the basis of pathogenicity and host range. *Map* is one subspecies that has been traditionally considered as only an animal pathogen (Harris & Lammerding, 2001). It is distinguished from other mycobacteria in that it needs exogenous mycobactin, an iron-chelating agent, because it does not produce it like other members of the family. Within the subspecies there are host-adapted strains such as bovine, sheep and goat. Differentiation needs reliable laboratory techniques, which are examining the phenotypic and molecular properties of an isolate (Taylor, 1951; Harris & Lammerding, 2001). Taxonomic placing of isolated strains on only phenotypic properties is laborious and not always accurate. Thus, analysis of strain genomes is necessary. However, even genome analysis could hardly either perform or successfully differentiate strains due to a high degree of genetic species homogeneity within *M. avium* (Dumonceau *et al*., 1997; Hermon-Taylor *et al*., 2000; Bernstein *et al*., 2003; Bull *et al*., 2003). Accurate detection of the various species and strain polymorphisms is necessary for the accumulation of epidemiological information helping in investigating the relationship between animal and human infections. Until this knowledge be-

comes widely available, veterinarians should look into measures needed for successfully preventing human infection. They will be most effective if the possible routes of transmission are identified.

PATHOGENICITY OF MAP AND POSSIBLE ROUTES OF TRANSMISSION

Paratuberculosis causes emaciation and reduced productivity due to loss of meat, milk and the presence of infertility (Merkal *et al*., 1975). Milk production may decrease up to 16% resulting in loss of income in access of $100 per cow and lactation (Benedictus *et al*., 1987) and an analogous monetary loss is observed among sheep (Juste, 1997). Thus, economic losses are such that make *Map* an economically important microorganism among animals. If it is proven that it is also involved, one way or another, in human chronic disease leading to reduced quality of life and even death, it will also become a very important zoonotic agent. Although paratuberculosis has been demonstrated in many animal species other than ruminants considered as the natural host for *Map*, only recently the infection of humans with this microorganism is intensively investigated.

The microorganism resists destruction in the environment surviving in manure for periods of time longer than nine months (Jorgensen, 1977). Survival of the microorganism in the environment ensures not only infection of newborns within the farm (Collins *et al.*, 1994), but also of new entries and possibly contamination of clean farms by fomites transfers (Sweeney, 1996). The microorganism has been isolated from the uterus and placenta of infected cows, the semen of infected bulls (Larsen & Kopecky, 1970) and the milk of infected animals and women (Merkal *et al*. 1975; Merkal *et al*., 1982; Naser *et al*., 2000; Grant *et al*. 2001; Grant *et al*., 2002; Grant, 2003). Infection of fetuses is possible, but venereal infection of female animals is believed difficult either because there appears to be an age-related resistance or the numbers of microorganisms in semen are too low for survival (Merkal *et al*., 1982). Susceptible animals are in their few months of life (Rankin, 1961; Merkal *et al*. 1975). Young animals are thought of being infected by the suckling of teats contaminated with fecal materials having the microorganism, and their infection continues with the grazing of contaminated pastures (Merkal *et al*. 1975; Chiodini *et al*. 1984; Hermon -Taylor *et al*., 2000). Thus, the most likely route of transmission to mammals is the fecal-oral. Hence, one should expect that human infection by *Map* is higher among those working or living closely with animals. However, prevalence of Crohn's disease among farmers is not significantly greater than that of other professional groups (Shivananda *et al*., 1996). This is usually interpreted as evidence against the hypothesis that *Map* plays a role in the development of Crohn's disease.

Infection of animals is not only age related, but also dose related (Sweeney, 1996) and the same should be expected in man regardless of outcome of infection with *Map*. After an efficient number of microorganisms are ingested, they reach the small intestine where Payer's patches become the portal of entry. Payer's patches are fully developed at the time of birth and progressively disappear explaining the highest susceptibility of young animals and, perhaps, man to this microorganism (Landsverk *et al*. 1991; Vantini *et al.*, 2004). Crohn's disease is usually identified in young individuals from

homes with good hygiene practices and having already a Crohn's sufferer (Parkes *et al*. 1997; Parkes *et al.*, 1998; Collins *et al.*, 2000; Bernstein *et al.*, 2003). This is interpreted as evidence that the disease is resulting from genetic susceptibility (Parkes *et al*. 1997; Parkes *et al.*, 1998; Harris & Lammerding, 2001) rather than a single microbial cause, such as infection by *Map*. To the view of some scientists, had Crohn's disease *Map* as its cause, it would have been most prevalent either among those lacking good hygiene or those coming in direct contact with the source of the microorganism (Shivanada *et al*., 1996). Others believe that persistent stimulus of various antigens coupled with genetic susceptibility could determine the appearance or not of Crohn's disease (Dechairo *et al.*, 2001; Satsangi, 2001; Watts & Satsangi, 2002). However, even if *Map* is not the actual cause of Crohn's disease, genetic susceptibility could predispose to various "triggering" factors, one of which could be *Map* (Harris & Lammerding, 2001; Bernstein *et al*., 2003; Bull *et al*., 2003). In such a case prevention of human infection with *Map* should be the best protection. This is possible if one considers the probable routes of transmission from animals to men.

ROUTES OF EXPOSURE OF MEN TO *MAP*

In theory, men could be infected either by direct or indirect exposure to the agent. Direct exposure is non-dietary and indirect is dietary.

Non-dietary exposure. Crohn's disease is often observed within families having more than one individuals affected (Parkes *et al*., 1997; Parkes *et al.*, 1998; Collins *et al*., 2000) leading to the hypothesis of genetic predisposition. Although the gene believed responsible for predisposing to Crohn's disease is found in only 8% of Crohn's sufferers (Abreu *et al*., 2002). Meanwhile other researchers give contradictory conclusions about the suggested loci coding for genetic susceptibility (Rioux *et al*., 1998; Varmeire *et al*., 2000; Dechairo *et al*., 2001). Evidence of some kind of genetic predisposition shouldn't, however, rule out a direct transfer of an infectious causative agent from person to person through the oral route, thus the disease affecting more individuals than one in a family. In the case of oral infection, feces having *Map* could be the main source for this microorganism, as the same is important in animals. Thus, scientists expect a higher prevalence of Crohn's sufferers among those people working with animals. The latter has yet to be observed or documented, and some hypothetical reasons why this is not observed are given later.

Dietary exposure. Map has been isolated from the milk of mothers suffering from Crohn's disease and the milk of animals with or without clinical paratuberculosis. Thus, the most likely route of transmission is dietary exposure of infant children, like young animals and milk is the main source of *Map* (Naser *et al*., 2000; Grant *et al*., 2001; Grant *et al*., 2002; Lund *et al*., 2002; Bull *et al*., 2003). Children consuming non-pasteurized milk should be the most vulnerable to *Map* infection. They are from poorer populations in developing countries consuming the product of their own animals. Nevertheless, unsystematically collected evidence shows that Crohn's disease is no more prevalent among them compared to developed people consuming pasteurized milk (Jayanthi *et al*., 1992; Shivananda *et al*., 1996). However, developing people become as susceptible to the development

of Crohn's disease as those in developed countries once they migrate in the West (Jayanthi *et al*., 1992). In this case, cultural or environmental factors, not excluding dietary habits, could be important triggering factors, (Harris & Lammerding, 2001). Thus, if *Map* plays a role in inflammatory bowel disease, milk consumption should be the most likely source of this microorganism, assuming of course failing pasteurization.

Pasteurization of milk has followed heat and time protocols effective against various important zoonotic microbes, which, however, may need lower pasteurization temperature and time than *Map*. Within the genus *Mycobacterium*, *M. bovis* is the species, which has traditionally been of concern to consumer safety. This species is inactivated in lower pasteurization temperature and time than other species, including *Map* (Chiodini & Hermon-Taylor, 1993; Grant *et al*., 1999; Lund *et al*., 2002). These standards change further in disfavour of consumers according to the number of cfu/mL of *Map* in the milk (Grant *et al*., 1999; Grant, 2003). The cfu depend not only on the clinical status of an infected animal, clinically or subclinically infected, but also on the extent of fecal contamination of tanked bulk milk and formation of microbial clumps. Thus, the prevalence of clinically or subclinically infected dairy animals in a farm or region, the number of such farms included in each batch of pasteurization and the hygienic collection of milk are factors determining the risk to consumers from *Map* (Grant *et al*., 1999; Lund *et al*., 2002; Grant, 2003).

Experimental pasteurization at various temperatures has shown that *Map* is recovered from some samples pasteurized at 65^{o} C for 30 min and 72^{o} C for 15 s (Chiodini *et al*., 1993; Grant *et al*., 1999; Maylan *et al*., 1996). These studies are confirmed by identifying *Map* from retail pasteurized cow milk using PCR and the microbe was also cultured from the cream of some of the same samples (Millar *et al*., 1996; Lund *et al*., 2002). Heat tolerance of different *Map* strains has been observed after heat treatment for up to 90^{o} C for 15 s but the microorganism did not survive pasteurization at 72^{o} C for 25 s (Grant *et al*., 1999; Grant, 2003). Thus, for *Map,* duration of pasteurization at 72^{o} C is more important than heat. Hence, even pasteurized milk could be source of infection for consumers. Contamination of bulked tank milk, number of microorganisms in that and method of pasteurization could determine effectiveness of pasteurization, the number of consumers affected and the eventual number of clinically infected people within a given population. Mass production and distribution of such milk increases the number of possible human cases explaining, in a away, the higher prevalence of Crohn's affected western peoples compared to those of other parts of the world, assuming that *Map* is one such cause of Crohn's disease.

In developing nations fewer people may consume milk from an affected animal due to reduced availability of mass-produced and distributed milk and reduced number of children consuming it. Pasteurized milk or not is also used for the production of cheese. Thus, *Map* has been recovered from cheese (Sung & Collins, 2000), which in the developed nations is also mass-produced and distributed increasing further the numbers of people at risk of infection. Although milk is the most likely source of infection for humans, the survival of the microorganism in the environment and in the manure for long periods of time (Jorgensen, 1977) could result in contamination of organi-

cally grown vegetables used for raw salads. Since there isn't any published evidence on that, such a possibility should be investigated.

Another source of *Map* for man could be utensils used in the cutting of contaminated meat and meat products or the cut surfaces of preparing such products. They could become indirect sources of contaminating other foods and utensils, thus becoming sources of human infection with *Map*. Further more, water has been discriminated as a source of infection, at least for immunocompromised individuals (Collins *et al*., 1984; von Reyn *et al*., 1994) and it should be investigated as either water for drinking, cleaning or food preparation. Infection of immunocompromised individuals could also be used as a model for explaining the mechanisms of genetic predisposition to Crohn's disease (Abreu *et al*., 2002) and the possible important role of *Map* or other microbial antigens to its development. Immunosupresive macrophages induced by phagocytosed *M. intrracellulare* transmit their suppressor signals to T lyphocytes (Shimizu *et al*., 2004). A similar mechanism could play a role in *Map* infection and bowel inflammation, as experimental infection of mice with *Map* has shown (Mutwiri *et al*., 2001).

CONCLUSIONS AND HYPOTHESES

1. The hypothesis that *Map* is involved, somehow, in the etiology of Crohn's disease, thus being an important zoonotic agent, has been extensively reviewed by other scientists. One such group commissioned by the EU (Shivananda *et al*., 1996), did not rule out *Map* as a possible cause of Crohn's disease, although genetic predisposition to the disease was also thought as possible cause (Abreu *et al*., 2002). Even, if genetic predisposition is required for the development of Crohn's disease, the possibility of *Map* being one of the triggering antigenic factors (Harris & Lammerding, 2001; Bernstein *et al*., 2003) should concern veterinarians. Thus, veterinarians should prepare for effective measures against a potential zoonotic agent that could be transmitted through consumption of foods of animal origin.
2. The aforementioned possibility is supported not only by the current knowledge, but also by those evidence used to rather disassociate Crohn's disease from *Map* than associate it. This evidence is based on the difference in prevalence of Crohn's disease between farmers and city residents and between developing and developed peoples (Jayanthi *et al*., 1992). However, the same evidence could also be interpreted as supporting an association.

If the above differences are interpreted differently, partuberculosis and Crohn's disease could appear as having a common cause. Specifically, lower prevalence of Crohn's disease among farmers could result from: 1. The low prevalence of *Map* infected animals within farms (Collins *et al.*, 1994) resulting in fewer people being directly affected by infected animals, few of whom are young individuals being among the most susceptible. 2. Fewer people are needed to attend farms than people consuming mass-produced infected foods. They are also of older age rather than young individuals having a higher risk in developing the disease and 3. City residents do not come in direct contact with infected animals or farms, but more people among the susceptible ages are at risk due to mass distribution and consumption of foods possible sources of

Map. For example, infected milk consumed unpasteurized, by the farmer's family and workers, puts at risk few people and even fewer when the age factor is introduced. In contrast, many more young people consume infected milk due to failing pasteurization. They are also consuming daily high quantities of milk as a drink, thus having an increased risk to infection.

As for the observed difference in the prevalence of Crohn's disease between developing and developed people, it could, perhaps, be more the result of a skewing diagnosis of Crohn's disease than other factors. The diagnosing of small intestine inflammation is difficult and even harder is the differentiation of various bowel conditions. The development of newer high technology diagnostic methods (Maggi *et al.,* 2003) is increasing the accurate identification of Crohn's disease or accurate differential diagnosis, but they are available only to wealthy western patients. This means that in developing nations either other health problems of the digestive track are shadowing Crohn's disease or the diagnostic means and experience are not available for an early accurate recognition of this condition. Therefore, sufferers are not recognized in developing countries as early as in developed, and many could eventually die from other causes. Possibly, the latter explains also the finding that once they migrate in developed countries they show the same prevalence as those of the host country. It could be that better opportunities of good health care result in diagnosis. In addition, if genetic predisposition exists, it appears that it is triggered by factors found within the host country.

On the other hand, the finding that Crohn's disease is more prevalent among men of Anglo-Saxon ethnic origin (Jayanthi *et al*., 1992) is not necessarily suggesting genetic predisposition. Such prevalence could also result from cultural factors. Perhaps, they either consume more milk, raw salads or cheese than others, all sources of possible food-born infection by *Map.*

Regardless of the factors contributing to the development of Crohn's disease, the role of subclinical disease or unapparent infections of man by *Map* should also be further investigated. If subclinical disease exists, its role in a later age in the development of bowel inflammations should be investigated. Thus, Crohn's disease like Johne's requires systematically collected epidemiological data involving both animals and animal products. They should be the result of concerted, long-term efforts among scientists of various biomedical disciplines using similar methodologies across wide areas, if a reliable epidemiological association or disassociation of the two diseases is to be made. To this end, identifying the routes of agent transmission to man helps the implementation of effective protection measures.

In summary, there is plenty to be answered and much more to be done to find answers to legitimate questions. Hence, veterinarians and others responsible for public health protection shouldn't wait for doctors' conclusions on the actual cause of Crohn's disease in order to take measures against a possible food-born scare. Preparing for it they should:

1. Intensify epidemiological investigations among animals concerning information on the prevalence of *Map* infection. These must be the result of collaborative research involving large regions;
2. Intensify epidemiological investigations in collaboration with doctors concerning the involvement of *Map* in

Crohn's disease or the extent of subclinical human infections;
3. Intensify the attempts of isolating *Map* in animal products such as milk, cheese, meat etc.;
4. Initiate investigations concerning the contamination of other foods of non-animal origin, such as fresh salads;
5. Intensify the investigations concerning the microorganism's survival in the environment including wild animals and their involvement in the spreading of *Map* infection to mammals.

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Correspondence:

A. R. Burriel
Mavromihali 154
Athens 111472, Greece
e-mail: elpida@rediffmail.com
Tel: 0030-6972-314842