# SUMMATION

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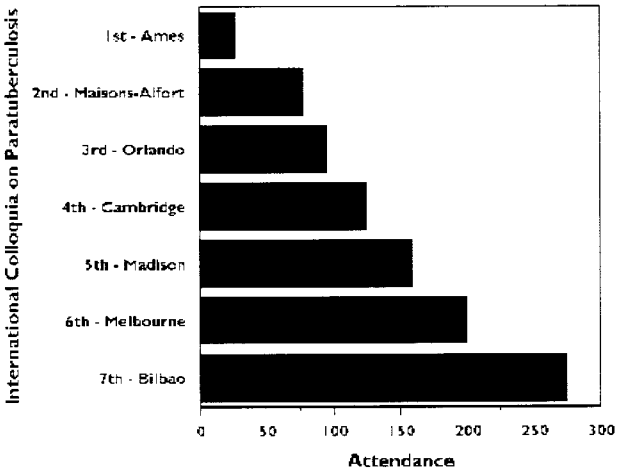
The 7th International Colloquium on Paratuberculosis was a great success thanks to the excellent organizational skills of Dr. Ramon Juste, his staff and the Organizing Committee, the caliber of the scientific presentations, the financial support of the sponsoring organizations, and the lovely venue in Bilbao. This was the largest colloquium in the history of the International Association for Paratuberculosis with 273 registrants. This meeting has shown steady growth in attendance since its inception in 1983 (Figure 1). Among the seven scientific sessions spanning the four-day meeting there were 102 oral presentations and 113 posters providing state of the art scientific information on every aspect of paratuberculosis.




The strength of these Colloquia its very specific focus on a single etiologic agent, *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), and at the same time the breadth of topics related to the disease it causes: everything from cow chips to *Map* micoarray chips was discussed at the 7th International Colloquium on Paratuberculosis. The growth of paratuberculosis as a field of scientific investigation presents a challenge for future Colloquium organizers. Most attendees want to be able to attend presentations on all topics concerning paratuberculosis and so are reluctant to see sessions scheduled in parallel. At the same time, everyone recognizes that four 10 hour days of scientific sessions is exhausting.

When we next meet in Copenhagen, Denmark for the 8th International Colloquium on Paratuberculosis, no doubt our Danish colleagues will have found a solution for this scheduling problem.

This summation attempts to glean the highlights of important issues from each of the seven scientific sessions. Failure to mention a specific topic or presentation should not be interpreted to mean it was unimportant. Capturing 215 papers in a single summation is difficult. While I have tried to spotlight findings presented at the 7th International Colloquium on Paratuberculosis, I focus primarily on the challenges for the future. (Each session of the Colloquium is dealt with individually but not necessarily in the order presented at the Colloquium).

## ETIOLOGY

Presentations on *Map* ranged from the molecular to the microbiological. One of the single most significant developments in the field of paratuberculosis is sequencing of the full genome. Dr. Vivek Kapur's invited lecture the Colloquium and nicely summarized what is known to date. Sequence analysis and comparison to *M. avium* has already identified several new molecular targets waiting to be exploited for improved diagnostics and vaccines (see Huntley *et al.*, Merkal Award Lecture, pag.72

in this proceedings). Aided by sophisticated techniques such as microarray analysis and proteomics the pace of discovery on *Map* at the molecular level will rapidly accelerate in the coming years. Likewise, several improved methods for distinguishing among strains of *Map* will provide refined tools for molecular epidemiology thereby helping to track and better understand relationships among infections in different animal species.

There is still much to learn about the life of this organism outside its host as illustrated by studies presented on the microbial ecology of the organism in soil and silage. The abilities of this tenacious pathogen to resist killing by heat, drying, freezing etc. need much more study if the ecology of this pathogen is to be understood and risks of infection from environmental sources are to be quantified. The papers by Schroen *et al.* on soil, pag. 10, and by Katayama *et al.* on silage, pag. 16, respectively, nicely demonstrated the complexity of interactions between physical and chemical factors on *Map* viability. This information is needed to develop on-farm control strategies and effective mechanisms to kill this pathogen when it contaminates animal feed.

What seemed to be missing from this colloquium were classic microbiologic studies of *Map* designed to delineate the pathogenetic differences of the organism in its many varied states. This organism can exist as vegetative cells, as dormant cells that have shut down protein synthesis (Sung & Collins, pag. 26, in this proceedings), persistent cells capable of surviving over a year in the environment without replicating, as viable but unculturable cells (Machackova *et al.*, pag. 191, in this proceedings), and some suggest a protease-resistant form when infecting humans. Each of these forms may have different biological properties; forms found *in vivo* and in the environment may be quite different from those found in laboratory cultures. These differences must be characterized lest we find that our research is devoted to laboratory artifacts and not the organism as it exists in nature.

The discovery of IS*900* in the early 1990s heralded a new molecular definition of *Map*. Definition of mycobacteria as *Map* based on phenotypic characterization, largely growth rate and mycobactin-dependency, was replaced with a genetic definition of this agent based solely on the presence or absence of the insertion element IS*900*. For the first time at a Colloquium the credibility of the genetic definition of *Map* was called into question. Kapur and others suggest that this genetic element occurs in strains of mycobacteria other than *Map* whereas Hermon-Taylor and colleagues are convinced that it is found exclusively in, and thereby defines, mycobacteria as being *Map*. If the genetic element is found in other organisms, the analytical and diagnostic impact of the finding for Johne's disease control and research must be assessed. Resolution of this controversy is crucial and requires open communication and collaboration among members of the International Association for Paratuberculosis.

Not only has the definition of "our" microbe been called into question, but there continues to be divergent views on the appropriate name for it as well. Of the oral presentations that used the organism name in their title, 30 used the name *Mycobacterium avium* subsp. *paratuberculosis* and 20 used the name *M. paratuberculosis* (2 found a compromise using *M. a. paratuberculosis*). At the 6th International Colloquium on Paratuberculosis (1996, Madison, WI USA) a debate was held on the issue of nomenclature (see pages 93-113 in the proceedings of that meeting). Thanks to the efforts of Karen Stevenson and Ramon Juste, you will find few other publications that so comprehensively summarize the biochemical, microbiological, and genetic comparisons of the putative *M. avium* subspecies. In spite of the eloquent arguments of these authors, the past seven years have seen little progress in standardization of nomenclature for this pathogen. Many have adopted the long and burdensome name of *Mycobacterium avium* subspecies *paratuberculosis* while our colleagues who study *Mycobacterium avium* simply call it that, not *Mycobacterium avium* subspecies *avium*. Moreover, our colleagues in the field of tuberculosis continue to use the designations *M. bovis* and *M. tuberculosis* for two pathogens that are as identical to each other as *M. avium* is *Map*. Clearly I have tipped my hand indicating my nomenclature preference. I hope that we have resolved this issue at least among the International Association for Paratuberculosis membership by the close of the next International Colloquium.

## PATHOGENESIS

The interaction of microbes that are uniquely well-adapted to intracellular survival and replication, such as mycobacteria, and phagocytic host cells of the animal's immune system up-regulates and down-regulations genes of many forms and functions. Exploration of these interactions has become possible through microarrays that contain hundreds to thousands of genes for both the pathogen and the host. The results from these complex gene-specific studies are often volumes of data that at times can be difficult to understand or translate into recognizable biological mechanisms. Layered on top of this complexity is the biological variation in strains of *Map*, the conditions under which *Map* is

cultured, temporal effects (stage of infection) and individual animal variation in immune response (different antigens are recognized by different animals). The net result is that the future holds the promise of huge amounts of data but some significant challenges in how it should be interpreted. The field of paratuberculosis research should keep close watch on the better funded work on *M. tuberculosis*, *M. bovis*, and *M. avium* for clues on the host-mycobacterial pathogen relationship.

## IMMUNOLOGY

This session was actually devoted to immuno-diagnostics, rather than to fundamentals of the immune response to *Map* infection (those papers were incorporated in the pathogenesis session). Summarizing the presented papers is not possible but one theme seems apparent: diagnostic laboratories have available reasonably good assay systems for detection of antibody in serum or milk, from most any animal species, and gamma interferon released from sensitized leukocytes. What we do not have are good antigens to use as the basis of these assays. If the sensitivity and specificity of immuno-diagnostic assays for paratuberculosis are to be improved, it will take fundamental studies on the antigens of *Map*. Among the many challenges in this scientific arena are: 1) different antigens may be expressed at different stages of infection, 2) antigens produced in vitro (and then used in production of test kits) may be different from those produced in vivo, 30 different animals (due to host genetics) may respond to different antigens, thus kits may need to contain multiple antigens, 4) animals infected in utero may never respond to *Map* antigens due to immuno-tolerance. Clearly the people working on immuno-diagnostics have their work cut out for them.

## *Map* CULTURE

The good news is that pooling fecal samples clearly is an effective strategy to limit cost without compromising diagnostic sensitivity. The bad news is that the ability of laboratories participating in an international proficiency test to recover *Map* from bovine fecal samples varies widely (see Whitlock *et al*, pag. 226, in this proceedings). For fecal culture methods to be used reliably on an international basis for animal trade, careful comparison of methods and culture media are essential. The long-range goal must be international standardization, proficiency testing, and even certification of laboratories for paratuberculosis diagnosis by fecal culture.

Automated liquid culture detection systems, other than the BACTEC system, made their appearance for the first time at the 7th International Colloquium on Paratuberculosis. The TREK and MGIT systems, adaptations of technology used for tuberculosis diagnosis in humans, appear ready to join the list of mainstream diagnostics for paratuberculosis. Liquid culture systems can more rapidly determine which clinical samples do not contain *Map*. However, for those samples that trigger a "signal" a secondary test to identify which microbe triggered the signal is required. The cost effectiveness of any liquid culture system must therefore be judged in terms of both speed and cost of the final diagnosis.

## DIAGNOSTICS IN GENERAL

This topic was not listed as a specific session. Rather, presentations and posters on diagnostic methods were scattered among several sessions. Richard Whittington, however, provided an invited comprehensive lecture that placed diagnostic tests in perspective. My summation of his message is: there are no perfect tests. We must learn to live with this fact of life and not wait for technology to deliver diagnostic perfection. Instead, we must use the tools at hand to diagnose, control and prevent paratuberculosis.

Debate and discussion on the accuracy of diagnostic tests for paratuberculosis will be improved if investigators first define just what they are attempting to diagnose: for example, clinical paratuberculosis, fecal shedders of *Map* organisms, prepatent infections or animals likely to have decreased productivity as a result of *Map* infection. The conflicts and confusion about the diagnostic sensitivity and specificity of tests for paratuberculosis, in my opinion, stem from the lack of a clearly stated case definition, that is: just what is the test trying to diagnose? If a case definition is explicitly stated in reports on test evaluation, then these studies can more objectively be judged and interpreted.

## MOLECULAR BIOLOGY

The focus of this session was largely molecular diagnostics. Fascinating technology with equally fascinating acronyms such as LCx, IMS-PCR, RT-PCR, LAMP, PCR-ELISA, and TaqMan PCR were among those described for detection of *Map* in clinical samples. As usual for such reports, the reported diagnostic sensitivity and specificity compared to standard fecal culture methods look promising (to investors?). What is lacking in most reports is an evaluation of assay cost and throughput capacity.

Molecular genetic methods for *Map* detection come at a price. Animal agriculture cannot always afford that price. For most sectors of

animal agriculture it is not enough to be "right" (100 % sensitive and 100 % specific) all of the time. It is more important that diagnostic tests generate useful diagnostic information at an acceptable cost in a reasonably short period of time. Diagnostic tests are used to make business decisions about animals used to produce food in a market with very slim margins. Hence, the cost per test is a crucial determinant in the utility of any diagnostic assay. Additionally, animals generally are kept as herds or flocks, at times in excess of 10,000 animals. This means that the cost of testing for paratuberculosis for the individual herd owner is multiplied by the herd/flock size yielding a price tag in the thousands of dollars. More importantly, it means that laboratories must be able to handle samples from hundreds of animals at a time. In my home state of Wisconsin as an example, we have 1,700,000 dairy cows. If only 10 % of our cows were tested only one time per year and the laboratory staff worked only five days a week (260 days per year - no vacations or holidays), we would have to test 654 samples per day, 5 days per week, 260 days per year. Most molecular biologists pale at the thought of even 10 samples per day.

## EPIDEMIOLOGY AND CONTROL

Methods for control of paratuberculosis emphasize animal husbandry techniques to limit infection transmission (mostly common sense) and use of diagnostic tests to detect and isolate or eliminate infected animals. It is hard to argue with these basic principles, particularly when they seem to be supported by computer simulation models (albeit only supported by the beliefs of those creating the models). Yet, there is a paucity of scientific data to demonstrate under field conditions that these principles actually work - or to what extent they work. On balance, the global scientific community may be investing too much money in work to understand basic biology and not enough to understand field applications of current methods for paratuberculosis control.

Vaccination as a method of paratuberculosis control was comprehensively reviewed by Ramon Juste in his invited lecture. He provided convincing evidence that many studies have demonstrated how existing vaccines can decrease *Map* shedding and increasing herd-life of cattle, sheep and goats. As with diagnostic tests for paratuberculosis, a communication problem among investigators seems to exist and it stems from their failure to first define what the goal of their method (vaccine) is, i.e., decrease fecal shedding of *Map*, limit adverse effects of infection on animal productivity, decrease *Map* infection rate of newborn animals. Additionally, the economics of the animal enterprise must be considered when deciding if a vaccine is effective. Producers of milk from commercial dairy cows may have different objectives from sheep producers. Breeders (producing animals for sale as breeding stock) may have different objectives or paratuberculosis control needs than those using animals for commercial production of food. A clear statement of purpose could lead to clarity of analysis and clarity of discussion in this debate.

In the bigger picture, paratuberculosis seems to be spreading geographically, among herds or flocks of animals and among animal species. Paratuberculosis decreases animal productivity on an annual and lifetime basis. Control of paratuberculosis for the economic benefit of animal agriculture is clearly evident. Whether control of paratuberculosis is essential for the health and well-being of humans is an even more critical question. The answer to this question will dictate whether society at large has an economic stake in the epidemiology and control of Johne's disease.

## PUBLIC HEALTH

Candidly speaking, the economic importance of paratuberculosis does not seem to justify the large investments being made by many countries for detection and control of the infection. On the other hand, if *Map* can infect humans, and if foods of animal-origin are the major vehicles of exposure for the public to *Map*, then paratuberculosis may rank near the top of the list of important zoonotic pathogens needing to be controlled and worthy of investment by animal agriculture, if not society at large. Resolution of this biomedical / food safety question is critical.

Those scientists who venture into this controversial scientific arena to investigate and report on the association between *Map* and human disease are to be applauded. The zoonotic question is not generally the purview of microbiologists or veterinarians. Rather, it is the responsibility of the medical scientific community to decide this issue. Once this seemingly simple scientific question is decided, veterinary medicine, animal agriculture and the food industry can then respond appropriately. Until then, the veterinary and agricultural communities remain in a quandary as to how to respond to this emerging animal health problem.

Studies on the effects of food manufacturing technology such as pasteurization on *Map* viability, presented at the Colloquium and elsewhere, indicate that *Map* is hard to kill or remove completely from foods. Debate will rage for a long time on the impact of methodological details of such experiments. Pasteurizer flow turbulence, the impact of sequential stressors such as heat and low pH, etc. on *Map* viability will always be a fruitful arena for research and debate

but it will not resolve the central scientific dilemma. The fact will remain that unless *Map* is accepted by the medical scientific community as a pathogen of humans, i.e., that it is a zoonotic agent (like all other mycobacterial pathogens), then it is possibly a waste of money and human resources, or at least premature, to examine questions related to food processing technology.

Scientists with data showing no association of *Map* and a human disease such as Crohn's disease seldom come to International Colloquia on Paratuberculosis. Proving the absence of association is far more challenging than proving a positive association and such "negative data" is rarely appreciated by the scientific community. Thus, any summarization of papers presented at this Colloquium is biased. Regardless, I will attempt some broad summary observations based in part on information presented at the Colloquium but also on relevant recent publications.

Improved diagnostic tools for paratuberculosis in animals have provided increased evidence of *Map* infection of humans. However, the strongest evidence comes from use of IS*900* based molecular probes (see Sechi *et al.*, pag. 326 in this proceedings). As the specificity of such probes is called into question (see Bölske *et al.*, pag. 261 in this proceedings), the issue of *Map* association with Crohn's disease or any other human malady becomes clouded. Clearly the specificity of IS*900* for *Map* needs resolution.

Association does not prove causation. Proof of a causal relationship between *Map* and a human disease is a very difficult task. Experimental challenge studies in neonatal primates seems one of the few avenues of research left untried.

A more pragmatic approach to the Crohn's question is simply to attempt therapy of Crohn's disease with drugs capable of treating *Map* infection. Small scale studies suggest some success with this approach (see Shafran *et al.*, pag. 324 in this proceedings) but large scale double blinded trials have yet to be reported. Even if such trials are attempted, there are two potential hazards in this approach: 1) there is very limited data on the antibiotic susceptibility profile of *Map* using a large range of antimicrobial drugs tested alone and in combination on a diverse battery of *Map* strains *in vitro* and *ex vivo* making drug selection problematic, and 2) if only a subset of Crohn's disease patients have *Map* involvement, then antimycobacterial therapy of a substantial proportion of such patients may not be warranted and lack of clinical improvement in such patients could obscure any beneficial effect of the treatment on those harboring *Map*. Therapeutic trials on a subset of Crohn's patients selected based on diagnostic tests for *Map* seems more appropriate, but how to devise diagnostic tests when so little is known about the nature of the host-pathogen relationship is yet another challenge.

The incidence of Crohn's disease is rising globally. While a putative gene conferring susceptibility to Crohn's disease has been described in roughly 15 % of patients, epidemiological studies increasingly incriminate some early childhood environmental trigger for Crohn's disease. *Map* remains the most prevalent pathogen capable of inducing chronic granulomatous inflammation of the intestine in a diverse array of animal species. Exposure of the general public to *Map* via food or water not only plausible, in some foods in some countries it has been documented to occur (see Grant *et al.*, pag. 300 in this proceedings). Of all scientific questions facing paratuberculosis' researchers, whether *Map* is zoonotic emerges as the one most needing resolution.