Mycobacterium avium paratuberculosis:

INFREQUENT HUMAN PATHOGEN OR PUBLIC HEALTH THREAT?

I. INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a soil microorganism and the etiologic agent of Johne's Disease (JD), a chronic and progressive enteric infection considered to be one of the most serious diseases affecting cattle [1] and other domestic and wild animals, including sheep, goats, elk, and primates. Clinical progression of the disease includes severe diarrhea and weight loss, and the affected animals eventually either die or are killed [2,3]. JD is prevalent in domestic animals worldwide; its economic impact in the U.S. alone is stunning, with an estimated loss of $1.5 billion every year [4]. One study estimated the prevalence of MAP in U.S. cattle to be 1.6%, with a significantly higher prevalence (2.9%) in the dairy cattle subset [5]. Studies in more localized herds of dairy cattle have produced even higher estimates; one estimated the prevalence of MAP in cattle in California to be 9.4% [6]. The problem is even more serious in other countries: a study in Denmark estimated the prevalence of MAP in the dairy cattle to be 47% [7,8].

Mounting evidence supports a role for MAP as an etiologic agent (although it may be one of several) of Crohn's Disease (CD), a chronic relapsing inflammatory human disease of the gut. Symptoms include persistent diarrhea, cramping, abdominal pain, fever, rectal bleeding, loss of appetite, and weight loss. Some of this evidence includes:

- JD in cattle, which is caused by MAP infection, greatly resembles CD in humans in terms of the physical manifestations of the disease.
- Gross pathology and histopathology of JD and CD are similar [9].
- One study of the intestinal biopsies of CD patients found that MAP was present in 92% of all CD patients tested, as compared to MAP in only 26% of patients tested with noninflammatory bowel disease [10]
- Clinical trials of antibiotics to treat CD suggest some clinical improvement of patients [11, 12-15]. Although there are also a number of negative published studies, it should be noted that many of those studies attempted to treat CD with antibiotics that are ineffective *in vitro* against *M. avium* and MAP [11].
- MAP has been cultured from the breast milk of women with active CD [16].
- MAP has been detected in the cultured blood of patients with active CD [17].
- One case report describes a boy with MAP cultured from draining lymph nodes in his neck who developed typical CD 5 years later [18].
- MAP that was cultured from human beings caused typical JD when fed to baby goats.
- Epidemiologic evidence suggests that CD is increasing in prevalence, not only in the US, but also Japan [19] and Denmark [21].

It is possible that MAP not only causes JD in a wide number and variety of meat and dairy animals, but that at least some cases of CD could be zoonotic in origin, caused by direct or indirect transmission of the active MAP bacteria from animals (particularly cows) to human beings. While direct transfer has not yet been demonstrated, there is evidence that an unexpected percentage of the milk supply is contaminated with MAP from infected dairy cattle. A study on the detection of MAP in retail pasteurized whole milk in the UK showed that 7% of the

samples tested were positive for MAP by PCR [20], while another study in Switzerland found that nearly 20% of milk in tested bulk tanks contained MAP [22]. Another study provided evidence that MAP in naturally infected milk survived commercial HTST pasteurization when the bacteria were present in sufficient numbers [23]. If a solid link is established between MAP infection and even some CD, the public health implications of a contaminated retail milk supply are enormous. It will be vital to develop new methods of preventing MAP infection and transmission, and new diagnostics and treatments for MAP infection in both cattle and human beings.

II. NEED FOR AND TIMELINESS OF THE PROPOSED MEETING

The American Academy of Microbiology (AAM) plans to convene a colloquium entitled **"*Mycobacterium avium paratuberculosis*: Infrequent Human Pathogen or Public Health Threat?"** As discussed in the introduction, we believe that MAP is an existing underappreciated and emerging potential public health threat, and deserves a comprehensive examination of its role in animal and human disease and an evaluation of the events underlying MAP transmission from animals to humans.

The link between MAP and CD has been hotly debated for decades: because of the importance of this topic, the NIH convened a workshop to evaluate the evidence for a link, and the report of that workshop was published in 1999. The conclusion from the workshop was that there was insufficient data to either prove or disprove a cause and effect relationship between the bacterium and the disease. However, since that workshop, a number of interesting scientific findings have reopened the debate and rekindled interest in solving the mystery of the underlying cause of CD. Among the recent findings that may shed light on a link between MAP and any human infectious disease (including CD) are:

- Microbial etiologies (or putative etiologies) for chronic diseases long thought to be induced by other non-infectious causes (ulcers, cardiovascular plaques, etc.) [24]
- Ability to identify MAP in biopsies of human patients with CD and its relative absence in normal controls [25].
- Identification of MAP in biopsies from CD patients by FISH [26].
- Identification of a gene that influences susceptibility to development of CD in ~15% of CD patients, CARD15/NOD2, a gene that influences the ability of humans to mount an immune response to a component of the mycobacterial cell wall [27]
- Discovery and cure by antibiotics of a CD patient with both NOD2 gene and MAP infection.
- Results from four open-label clinical trials of antibiotics for the treatment of CD that demonstrated between 50-75% efficacy

Other meetings have been held on issues related to MAP, but no specific reports have emerged providing an objective analysis of the possible public health hazard represented by MAP. It is important to identify the gaps in our knowledge, and to focus new research to resolve these questions. We therefore believe that our report will fill a need for credible research recommendations for the future.

III. OBJECTIVES AND PRINCIPAL TOPICS TO BE COVERED

The AAM colloquium format is unique. The goals are to identify key disciplines and experts who, through working sessions over a 2 ½ day period, will synthesize the current status of the science and develop a report which can be used by government agencies, scientific and lay

communities, the public, and academics to answer critical questions of our time on particular topics.

The AAM will convene a colloquium—defined by Webster as “a discussion meeting”— of international scientific experts to develop the intellectual material for a written report. These experts will gather to consider each topic in a setting and format that encourages open and vigorous discussions. These deliberations will form the foundation for a report, published by the AAM, that will include a succinct description of the issues, graphical representations, where appropriate, and recommendations for future action.

The steering committee has composed a set of preliminary questions, intended to be the focus of the colloquium, and these are listed below. The bulk of participants’ time will be spent in small working groups addressing each of the questions that the steering committee has developed in advance. The colloquium is highly structured; however, there is sufficient time and flexibility for creative and spontaneous exploration of the issues. It will be made clear that these questions are the starting point for discussion; the groups will be free to explore issues as they arise in the course of deliberations.

Environmental/zoonotic sources of MAP and control measures

1. What is the prevalence of JD in cattle, both in the U.S. and globally?
2. What other domestic meat or dairy animals are infected with JD?
3. How is JD diagnosed currently, and are there new diagnostics in development? How accurate are these diagnostics?
4. What are the measures used in the control of JD, and how successful are they? Should animals who test positive for JD be killed and culled from the food chain?
5. What is known about the prevalence of MAP in the milk supply and its viability following pasteurization, both in the U.S. and globally? Does ultra pasteurization kill MAP any more effectively than regular pasteurization? What other strategies could be investigated to kill MAP in milk? What evidence is there that this is a source of MAP infection in humans?
6. Is there evidence for the transmission of MAP from cattle outside of the milk supply, or from other domestic animals?
7. What are the other zoonotic or environmental sources of MAP that could contribute to the spread of infection in humans? How are these controlled?
8. What is the prospect of finding a vaccine for MAP that could prevent transmission among animals, and possibly prevent the development of active CD in individual people who are genetically susceptible to MAP infection?
9. What is the evolutionary logic of host-parasite interactions as causes of chronic disease, as opposed to autoimmune mechanisms?

Human MAP infection

1. What is the prevalence of MAP infection in humans?
2. What are the known/possible pathologies of MAP infection in humans, and what is the evidence for a direct link to MAP?
3. What are the methods of diagnosing MAP infection in humans, and how sensitive and specific are they?

4. What new laboratory techniques could be employed to detect MAP accurately in humans?
5. What are the methods of treatment of MAP infection in humans?

Potential role for MAP in CD

1. What methods are used to diagnose CD?
2. What treatments are used in CD, and how effective are they?
3. What are the side effects and risks of current approved treatments for CD?
4. What is known about genes related to susceptibility to CD?
5. What is the evidence for and against a role for MAP in CD?
    a. Are immune responses specific to MAP consistently reported in CD?
    b. Do tissue samples from CD patients contain MAP?
    c. What are the similarities and differences between the pathologies of CD and JD?
    d. Have antimycobacterial drugs been successful in the treatment of CD, and if so, is there a correlation to activity against *M. avium* and/or MAP?
    e. What are the side effects and risks of antimycobacterial treatments for CD?
    f. Is there a correlation between incidence of CD and potential sources of MAP infection?

Gap Analysis

What additional information/research is necessary to further clarify the role of MAP as a human pathogen?

IV. LOCATION AND DATES OF COLLOQUIUM

The colloquium will be held June 15-17, 2007 in Salem, Massachusetts. This location has been selected because of its proximity to an international airport and reasonable hotel prices.

V. ORGANIZATION OF COLLOQUIUM, OUTCOMES, AND CONTRIBUTIONS

A group of 30-40 scientists with a broad range of expertise (ranging from basic science to clinical medicine, veterinary biology to human disease) will be invited to participate in this 2 ½-day colloquium. Attendance at the colloquium is by invitation only, and participants are selected to ensure the greatest scientific balance and diversity. The AAM, through funding supporters for this project, will provide travel support for colloquium participants, including airfare, ground transportation, on-site lodging and meals, and other expenses related to attendance.

The bulk of participants' time will be spent in small working groups addressing each of the questions that the steering committee has developed in advance. The colloquium is highly structured; however, there is sufficient time and flexibility for creative and spontaneous exploration of the issues.

The general sessions will bring all colloquium participants back together to share the working group conclusions and recommendations and discuss any issues raised. Following the colloquium, a science writer (who will attend the colloquium), working closely with the steering committee chair, will develop a draft report for review by colloquium participants.

The agenda for the colloquium is as follows:

Day 1

	Participants arrive; no scheduled events

Day 2

7:30-8:30 am	Group breakfast and information on scientific discussions
8:30-10:00 am	Welcome, introductions, and panel presentation of the issues
	Charge to participants
10:00 am-12:30 pm	Working groups
12:30-1:30 pm	Group luncheon
1:45-5:30 pm	Working Groups
6:30 pm	Group dinner and information scientific discussions

Day 3

7:30-8:30 am	Group breakfast and informal scientific discussions
8:30 am-12:30 pm	Working groups
12:30-1:30 pm	Group lunch and informal scientific discussions
1:45-5:30 pm	General session
6:30 pm	Group dinner and informal scientific discussions

Day 4

7:30-8:30 am	Group breakfast and informal scientific discussions
8:30 am-12:00 noon	General session
	Working group presentations
	Final remarks
12:00 noon	Adjourn

Following the colloquium, our science writer will draft a report, summarizing the deliberations of the working groups. The draft report will be sent to all colloquium participants for review, followed by peer-review by the AAM's Board of Governors. The report will then be published and posted on the AAM's web page. A specific outreach plan will be developed, including:

- Press release announcing the report will be sent to relevant scientific publications, such as *Science, Nature,* as well as to popular science publications, e.g., *Science News, Scientific American, Discover, Popular Science,* and *New Scientist*.
- Press release will be posted on EurekAlert, the web site for science journalists hosted by the American Association for the Advancement of Science (AAAS).
- The report will be posted to the web site for the American Society for Microbiology (ASM) and announced to the 43,000+ members of the Society through the "What's New" section of the Society's home page (http://www.asm.org). To date, there have been over 460,000 downloads of AAM colloquia reports.

- An email announcement will be sent to all Fellows of the AAM, as well as members of relevant scientific divisions of the Society.
- Copies of the report will be provided to the leadership of the ASM, colloquium supporters, colloquium participants, and member organizations of the International Union of Microbiological Societies.

Government agencies, industry, educators, and the scientific and lay communities have a strong need for objective, credible analyses, assessments, and recommendations on critical issues in microbiology. AAM colloquia are designed to evoke just such information. Our reports are viewed as unbiased statements of the issues and practical recommendations for the future.

Contributions

The predicted contributions to the enhancement and improvement of science are:

- Objective analysis of what is known about *M. avium paratuberculosis* as a public health problem; and
- Recommendations for future research to clarify the gaps.

VI. STEERING COMMITTEE

Members of the steering committee were selected as representative of the various stakeholders with interest in the issue of *M. avium paratuberculosis* as a potential public health threat, including infectious disease experts, practicing gastroenterologists, veterinary sciences and food safety experts, and the CD patient population.

Chair: Carol A. Nacy, Ph.D., Sequella, Inc, Rockville, MD

Marcel Behr, M.D., Ph.D., Division of Infectious Diseases and Medical Microbiology, McGill University, Montreal, QB, Canada

Charles Bernstein, M.D., Inflammatory Bowel Disease Clinical and Research Centre, University of Manitoba, Winnipeg, MB, Canada

Judith Eve Lipton, M.D., private practice, psychiatry; Distinguished Fellow of the American Psychiatric Association, and a patient with Crohn's Disease, Redmond, WA

Mary E. Torrence D.V.M., Ph.D., DACVPM, National Program Leader, Food Safety, USDA, CSREES, Washington, DC

VII. RECRUITMENT OF PARTICIPANTS AND SUPPORT

Colloquia sponsored by the AAM differ significantly from traditional conferences in which formal presentations are made. Colloquia have no formal presentations, and honoraria are not provided.

All invited participants are assigned to working groups. The approximately 30-40 invited scientists represent the range of interdisciplinary approaches that can be applied to the issues. We have included a number of younger, but proven, scientists in our list of potential participants for their fresh perspective and to provide them the opportunity to deliberate with more senior scientists. It is critically important to have key international scientists to facilitate harmonization of goals and strategies from other parts of the globe.

The steering committee carefully selects each participant, giving all due consideration to including underrepresented minority and women scientists and scientists with disabilities. The

committee develops the intellectual approach to the issues, including drafting the questions to be deliberated at the colloquium. Then the committee determines the scientific disciplines that must be represented to maximize discussion and expertise, and from this list of disciplines the committee develops the final list of participants upon consultation with the Academy's Committee on Diversity. The Academy's Committee on Diversity's mission is to increase the visibility and participation of underrepresented minority and women scientists in the Academy and its programs. One of the ways in which the committee achieves its mission is by direct participation in identifying scientists with appropriate expertise as potential colloquium participants.

Potential Participants

The steering committee has prepared a preliminary list of potential participants from both academics and industry, with interest and expertise in different areas including MAP microbiology, diagnosis, treatment, and human and animal health.

1) Dr. Peter Anderson, Statens Serum Institute, Denmark (mycobacterial cell-mediated immunodiagnosis)
2) Dr. John Bannantine, USDA Iowa (genomics of MAP)
3) Dr. Ed Boedecker, University of New Mexico (MAP in CD)
4) Dr. Tom Borody (formerly associated with early *H. pilori* studies, clinical trial of CD with antibiotics)
5) Dr. Sheldon Brown, Bronx VA (anti-mycobacterial drugs and MAP susceptibilities)
6) Dr. Will Chamberlain, Texas Tech, (MAP in CD)
7) Dr. Rod Chiodini (MAP immunology)
8) Dr. Robert Clancy, Univ. of Newcastle, Australia (MAP expert)
9) Dr. Michael Collins, University of Wisconsin (Johne's Disease)
10) Dr. Paul Coussens, Michigan (immunology of MAP)
11) Dr. Jay Ellingson, Marshfield Clinic (author: MAP in 2% of retail pasteurized milk from WI, MN, CA)
12) Dr. Brian Geisbrecht, University of Kansas (MAP proteins)
13) Dr. Leonid Heifets, National Jewish, Denver (Antimycobacterial agents)
14) Dr. John Herman-Taylor (2003 publication: 90% of Crohn's Disease patients had MAP in biopsies)
15) Prof. Richard Hunt, McMaster University, Canada
16) Dr. Vivek Kapur, University of Minnesota (Network coordinator, Johne's disease)
17) Dr. Mark Klassen, Canadian Beef Export group
18) Dr. Preston Linn (Alliance Manager/Business Development, BD Technologies)
19) Dr. Elizabeth Manning, University of Wisconsin (MAP pathogenesis)
20) Dr. Saleh Naser, University of Central Florida (MAP genome)
21) Dr. M. Netea, Nijmegen, the Netherlands (NOD2 gene as *Mycobacteria* sensor)
22) Dr. Norman Pace, Colorado (M. avium 16s identification)
23) Dr. Jim Rothel, Cellestis (Quantiferon testing)
24) Dr. David Russell, Cornell University (intracellular pathogens, including *Mycobacteria*)
25) Dr. Katherine Sacksteder, Sequella, Inc. (MAP diagnostics)
26) Dr. Balfour Sartor, University of North Carolina, (MAP in CD)
27) Dr. Fergus Shanahan, National University of Ireland (MAP microbiology)
28) Dr. Christine Schewe (validation of PCR-based detection of *Mycobacteria*)
29) Dr. Salman Siddiqi, Becton Dickenson, (*Mycobacteria* expert)
30) Dr. Christine Sizemore, National Institute of Allergy and Infectious Disease, NIH (Program Officer of TB, Leprosy and other Mycobacterial Diseases Branch)

31) Dr. Hong Tang, National Institutes of Health (organizer of past NIH/Crohn's Disease workshop)
32) Dr. David Taylor, Salix Inc. (formerly of Johns Hopkins School of Public Health)
33) Dr. Michael Towns, Medical Director Becton Dickenson
34) Dr. Richard Whittington, Australia (MAP diagnostics, in sheep)
35) Dr. Lee Ann Jaykus, NC State (food microbiologist)
36) Dr. Garry Adams, Texas A & M University (Johnes coordinated agricultural project)
37) Dr. Yrjo Grohn, Cornell (Epidemiologist)

The AAM through its funding supporters, will provide travel supports for colloquium participants, including airfare, ground transportation, on-site lodging and meals, and other expenses related to attendance.