NIMBIOS MAP modeling meeting, March 4-6, 2013 in Knoxville.

Monday:

9:00	welcome and introductions, summary of phone conferences, key point meeting I
9:00-12:00	Report on modeling efforts: team 1 – Tennessee team
13:00 – 15:00	Report on modeling efforts: team 2 – Don and Ad
15:30 – 17:00	Report on modeling efforts: team 3 – Yoram
7:00	Dinner

Tuesday:

9:00 – 11:00	Report on review paper MAP biology – Ad & Sri
11:00-12:00	Define afternoon plans
12:00 – 1:00	Lunch
1:00-5:00	Teams to work on models – map biology paper
5:00-6:00	Teams report progress
7:00	Dinner

Wednesday:

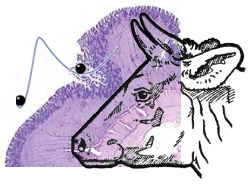
9:00-10:00	Continue progress reporting
10:00-11:00	Discuss possible funding opportunities for the next phase
11:00-12:00	Develop plans, timelines and responsibilities
12:00	Lunch and farewells

Each of the three model teams will prepare a presentation and lead the discussion on their respective models. Each team will ask for input, data and suggestions that are needed to improve the models.

Ad and Sri will present the literature review outline and ask for for input, data and suggestions that are needed to improve and complete the paper.

Objectives of the meetings as previously defined:

a. The first meeting will be used to make an inventory of existing knowledge of MAP immunology and pathogenesis, to develop an initial set of mathematical models.
b. The second meeting will be used to finalize a mathematical model for MAP infection within host and to understand the biological implications of the modeling results. The second meeting will also used to start a series of manuscripts on the results of the working group.
c. The final and third meeting of the working group will be used to use the developed mathematical model of the within host dynamics of MAP to perform simulations and develop predictions.



Working Group Within host modeling of *Mycobacterium avium* subsp. *Paratuberculosis*
Summary of first meeting, June 26 to 28, Knoxville, TN.

Present: Drs. Eda, Klinkenberg, Koets, Lenhart, Louzoun, Magombedze, Martcheva, Mitchell, Momotani, Schukken, Sreevatsan and Stabel and guests (see below).
Absent: Drs. Zhou and Coussens

The first meeting of the working group on within host modeling of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) met from June 26 to June 28, with 11 of 13 working group members present. One member had a conflict (Dr. Coussens) and one member had a family emergency that came up the day of the first meeting (Dr. Zhou). Also, present at the meetings were several guests from the NIMBioS program: Drs. Ngonghala, Ganusov and Agusto. Several NIMBioS REU students (Johne's disease epidemiology modeling group) also joined the meeting at various points in time. The REU students had a chance to informally talk with some of the working group members during lunch and coffee breaks.

The first day of the meeting was dedicated to presentations of the members of the working group. A total of four presentations of the current knowledge on immunology and pathogenesis of MAP infections were presented by the members of the group. Subsequently four presentations on modeling principles and initial MAP infection dynamic models were presented by the members. Finally two presentations on existing data sets to be used for the modeling process were shown by members of the working group.

The group discussed the current knowledge, the gaps in current knowledge and the direction for the working group over the next days. It was decided to focus effort in three areas: 1) develop a consensus biological framework for infection progression and pathogenesis of MAP infections in the bovine; 2) develop an inventory of available datasets that can be used by the working group to validate the immunological models that will be developed by the working group; 3) develop a series of mathematical models for within host dynamics of MAP infections with a strong immunological basis.

1) Consensus biological system for infection progression of MAP

The infection process of MAP as far as is currently known consists of an initial entry of MAP through the M-cells that are present in large numbers in the young animals covering the Peyer's patches in the ileum or entry takes places through enterocytes in older animals. The fate of the MAP cells after entry into the epithelial lining is baso-lateral expulsion and uptake by sub-epithelial macrophages and dendritic cells or FDCs. In infected macrophages, the MAP cells will replicate intracellularly. Infected macrophages may eventually undergo apoptosis and apoptotic bodies with MAP cells (or free MAP cells) will be taken up by the next generation of infected macrophages and the cycle will continue. The process of years of continuous replication and infections of generation of macrophages with only limited activation of macrophages, pro-inflammatory immune response and virtually no evidence for adaptive immune response is not fully understood. In a proportion of animals this cycle of infection with limited immune responsiveness of the host is interrupted and an adaptive immune response is mounted as evidenced by an increasing antibody titer against MAP. Infected macrophages may organize in granulomas and these

intestinal disturbances will eventually result in clinical Johne's disease. The importance of lymphocytes (T-cells and B-cells) requires further understanding. The process of cell migration and its role in the development of multiple granulomas over time is not fully understood. The start of and the role of adaptive immunity is not clear and the role of antibodies in bacterial killing requires elucidation. The importance of extracellular cellular bacteria in modulating immune response (stimulation of Th2) and potential killing of the bacteria by anti-MAP antibodies needs further discussion and histo-pathological studies. The triggers that lead in a proportion of animals to development of granulomas and clinical disease are unknown. Bacterial shedding may happen throughout the infection process but is only present or detected in a relative small proportion (~10%) of infected animals. Drs. Koets and Sreevatsan will lead an effort to develop a position paper on current immune-pathological understanding of MAP infections in the bovine. The objective of this part of our work is to complete a manuscript for submission to a well-recognized journal.

2) Inventory of available datasets

An inventory of datasets available to the members of the working group resulted in a rich list of datasets: Dr. Sreevatsan has data on in-vitro intracellular cell count; Drs. Mitchell and Schukken have a 7 year longitudinal dataset on three dairy farms; Dr. Momotani has National Japanese data from infected farms and a 5-year longitudinal dataset of experimentally infected animals; Dr. Stabel has longitudinal data on known infected animals, including immunological parameters and tissue infection data; Dr. Koets has data on a 5-year follow up experimental map infection in 20 animals with monthly immune data and fecal PCR and antibody ELISA data. Dr. Louzoun will ask for MAP ELISA data from 200 Israeli dairy farms, this dataset also has all animal movements between farms; Dr. Eda has 5 year longitudinal data on animals with an experimental MAP infection. Dr. Schukken will also ask for the availability of datasets from Drs. Whitlock and Sweeney. The objective of this effort is to have all datasets available to the working group before the next meeting in March 2013.

3) Series of mathematical models for within host dynamics of MAP

The existing models were discussed and it became clear that a series of next generation models will be important to better understand the within host infection dynamics of MAP. Publications (and computer program codes) coming out of these first generation models will be made available to the working group members. The ultimate goal of the next generation modeling effort is to understand what leads to perturbation of the steady state situation such that the MAP infection process is out of control and results in high bacterial shedding and intestinal granulomas. The first specific objective for the next generation of mathematical models is to closely connect immunological understanding of the MAP infection process with the mathematical modeling of the infection process. The objective of the first model to be generated is to recreate a steady state situation, where the main components of the infection and immune response process are incorporated in the model in such a way that a long term steady state exists. This steady state of MAP infection and host immune response that does not result in long-term pathology is the infection situation that is present in the majority of MAP infected animals. The main components of the infection and immune response process were defined as MAP bacteria (intracellular and free), resting and activated macrophages, key cytokine (IL-1α, IFN-γ, IL-10, IL-4) profiles, lymphocytes (B and T cells) and antibodies against MAP. It is expected that this generation of

models will have a stochastic component. Drs. Louzoun and Klinkenberg will lead the effort to develop an initial conceptual model.

The next meeting of the working group will be from March 4 to 6, 2013. Monthly conference calls to discuss progress will be held on the first Tuesday of every month at 9 AM EST starting on August 7, 2012. The members absent at the meeting and Dr. Whittington will be invited to the monthly conference and future meetings.