The Paratuberculosis Newsletter

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Editor

1. Comments & Opinions

An Epidemiological "Gold Standard" or "Fool's Gold"

Gilles R. G. Monif, M.D.

Culture identification of *Mycobacterium avium* subsp. *paratuberculosis* (Map) has long been considered the "gold standard" for basing epidemiological data (1-3). Justification for this contention resided in the fact that the prevailing Map ELISA tests, while having a positive correlation between positivity and the ensuing development of clinical manifestations (Johne's disease), lack both the antigenic spectrum and sensitivity required to identify the entire pathogenic spectrum.

What a positive culture confirmed using the IS*900* insertion sequence, establishes is that an animal is infected with an IS*900* identifiable Map isolate. The positive establishment of infection is a very valuable piece of information specific to that animal, but epidemiologically, it represents only a small, limited vertical cut into what is a broad and complex horizontal spectrum.

In the necropsy files of the University of Florida College of Veterinary Medicine, 24 beef and dairy cows with the histopathological diagnosis of Johne's disease were identified that had corresponding fecal samples. Using the broad spectrum IS1311 direct and nested IS*1311* primers (FecaMap®), the fecal samples were analyzed for the presence of DNA encompassed by pathogenic mycobacterium in the Ma variant/Map spectrum. Using only the direct IS*1*/IS*2* primer only 10 of the 24 diseased cows tested positive. The IS1/IS2 primers minimum identification level is between 10 to the 5th - 6th cfu. Using FecaMap's nested primers that can identify approximately one log of targeted DNA, 19 of the 24 cows tested positive.

Why not 24 out of 24? A possible explanation is that of biological DNA degradation over time. That only 1 of the 7 four-year-old fecal samples tested negative casts doubt on biological degradation as the reason for negative PCR test results.

Incomplete antigenic coverage is a possibility. The IS*1311* primers were specifically developed to address the spectrum of pathogenic mycobacteria that can produce chronic granulomatous disease of the bovine gastrointestinal tract. In comparison with direct and nested IS900-based primers on known Map isolates within three USDA's

Laboratory Certification Tests, the IS*1311* direct and nested primers demonstrated superior sensitivity. (4). Added intraspecies genomic variability within Map resulting in incomplete antigenic coverage is a valid possibility.

A third explanation is that Map fecal shedding in animals with far advanced disease, is intermittent. This possibility is difficult to analyze without the availability of serial fecal samples.

A more plausible explanation is that of sample error. In collaboration with Dr. Ching Ching Wu at Purdue University, we looked at the degree to which clumping by Map might engender an erroneous assessment of Map presence (5). In a double-blinded prospective study, fecal culture, hspX real-time PCR and direct and nested IS1311-based PCR testing for

Map had been compared. The three methods used to identify Map required separate test samples being taken from a given fecal specimen. Only 7 of the 22 samples identified as coming from "heavy shedders" as identified by culture had positive correspondence with real-time and nested PCR. In 8 instances, neither the real-time nor nested PCR tests were

positive. The non-concurrence between the three tests argues for non-uniform distribution of organisms within feces and the probability that sample error can occur.

The converse of sample error also impacts on epidemiological results and herd management recommendations. Quantitative assessments of the amount of Map present have been used to develop herd management recommendations. The presumption has been made that heavy Map representation in feces is predicative of advanced disease and hence, constitutes a defining criteria for culling a given animal from the herd (6).

Much of our epidemiological data, directly or indirectly, is based upon the fecal "gold standard". The "gold standard" label for identification of infection by an IS*900* Map strain for a specific animal is valid, but when used to establish a reference basis for epidemiological studies, the fecal"gold standard" may well be fool's gold.

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Welcome back Rod!

Ramon A. Juste

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Tradition has it that when XVI century Spanish friar Luis de Leon re-started his classes at the University of Salamanca after spending some time of Inquisition-forced retirement, he began his lecture with: “As we were saying yesterday…” It was really good news to see that our first President is back in the paratuberculosis business (see The Paratuberculosis Newsletter March 2010) and picking up the old thread. Although he never lost track of the field, his lack of direct involvement with the IAP was a definite loss for the field of paratuberculosis and other intestinal diseases. Welcome back Rod Chiodini!

Rod’s coming back could not be neutral, and he has greeted us presenting his next challenge. This challenge has been in the air for more than twenty years in a very specific form: “Is Crohn’s disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP or, as Rod prefers, just *Mycobacterium paratuberculosis*)?” I think Texas Tech University Health Sciences Center has done the sensible thing by opening a specific line of research on this question. Recruiting Rod for this purpose was also the right decision, given that it was he who made a scientific question of an old observation and started a debate that has been running, more or less heatedly, since then. Rod not being involved during these years is probably one of the main reasons that explain why no conclusions have been reached yet.

I avoided taking sides on this issue until I had the opportunity to work on the subject. It was a serendipitous confluence of circumstances that took us to a set of human blood samples for detecting MAP in collaboration with a medical institute. Just after having the project approved, this medical institute changed strategies and downgraded its priorities on Crohn´s disease. As a consequence, we also had to change the focus of the study and get more involved in the exploitation of the results. Because the results turned out to be the opposite of what we expected, we tried to find out if we could fit them in our concepts on paratuberculosis and the pathogenesis of other ruminant infections. We realized that we needed a model that did not comply with the classical concepts of causality attribution defined by Koch but that was not biologically impossible. This model has been published (Juste et al, 2008; Juste, 2010), and it is up to the scientific community to prove or to disprove it on the basis of its predictive utility. Here, I will just say that I now support a common causality for the general physiopathological phenomenon of inflammatory bowel disease (IBD) throughout species that, however, might take different forms even within the same species (Perez at al., 1996; Corpa et al., 2000; Gonzalez et al., 2005). From this perspective, I would like to discuss under a heterodox point of view, some of the key points that Rod has raised for the demonstration of causality in the MAP-IBD system.

Rod’s approach is the orthodox one and fully in line with established microbiological views. However, I think he overlooks some pathological and epidemiological perspectives that might have higher chances of breaking ground and opening up a new path to the demonstration of IBD causality. Trying to answer the inflammatory quiz with old tools is unlikely to work as the failure of brilliant researchers using relatively large resources and energies to come up with a solid answer has proven.

Rod's hypotheses are mainly and soundly based on Koch's old postulates. Koch's postulates were revolutionary in their time and have allowed the attribution of causality to many diseases, thus breaking the way to their cure. However, the same disease that inspired him does not actually work according to these principles, and it is clear that many diseases do not fully comply with them. Tuberculosis itself only causes disease in a small fraction of the susceptible individuals that are exposed to the causative agent and that actually carry it. It is estimated that only 1 in 218 (Corbett et al., 2003) of the infected humans will ever become a clinical case. If this is the situation for tuberculosis, why should IBD/paratuberculosis be different? Do not we often use the iceberg model for ruminant paratuberculosis? If we look only to an association under this classical Koch postulate it is unlikely that we will prove causality for IBD. What we really need is to see the problem under a new paradigm. I already proposed one: the slow infection model. This model is not that new and is implicitly assumed for many diseases. Actually, this general concept has been recently revised for Crohn's disease although surprisingly skipping MAP (Casanova and Abel, 2010). It is a particular case of multifactorial disease where a low virulence microorganism and a genetic profile with a relatively small, non-lethal, immune defect interact leading to a protracted course generally with a low disease to infection rate. The model was proposed over 50 years ago by B. Sigurdsson (Sigurdsson, 1954) for maedi and has not had a wide explicit use later. Coincidentally, was not the entrance of paratuberculosis in Iceland part of the same event?

As Rod rightly points out on question 1, a classical association of MAP with Crohn's disease has been energetically sought to find only fragmentary evidence on its support. In the majority of studies, MAP is not found in the intestinal tissues in higher frequency in Crohn's patients than in healthy controls. Our own studies actually show that MAP DNA is less frequently found in blood of patients than of controls. However, few studies have been carried out in non-treated patients. On the other hand, pathologists are familiar with the concept that in ruminant paratuberculosis paucibacillary forms, MAP is difficult to find even without any treatment. Therefore, I think it should be considered that the quantitative approaches proposed in Rod's first and second questions might fail to help in demonstrating causality because of the small numbers of bacteria involved in the animal forms with histopathologic lesions that mimic those in human IBD. The third and fourth questions deal with reproducibility of the association of MAP with the clinical form of the infection and association with a form of disease. Implicit concepts in these questions have the same limitations as the previous two questions. They do not take into account the subclinical infections (which are included as healthy controls from a clinical point of view) and once again the different amounts of bacteria present in the multibacillary and the paucibacillary forms of paratuberculosis.

It is my opinion, that the best way to find a causal relationship is defined in Rod's question 5, although, I would add, in a different non-quantitative perspective. We must define a role for MAP but in the context of a new pathogenesis model. In this sense, the genetic background of the individual is as important as MAP. In Koch's postulates underlies the concept that all individuals have the same susceptibility to the infectious agents. This has been proven not to be true. Susceptibility to infectious agents varies largely, and this is especially true for mycobacteria. We even now know of the case of Mendelian susceptibility to mycobacteria where a qualitative immune trait, which does not seem to affect other infections, becomes lethal when the carrier is exposed to environmental mycobacteria (Casanova, 2000). It is obvious that paratuberculosis does not behave this way, but it is also

pretty evident that genetic factors play an important role in human IBD and, as we and others are currently showing, also in ruminant IBD. Even though it is likely that strictly microbiological factors might play a role, I would like to bring up the accumulation of negative evidence on experimental infection success that was firstly pointed out by Rod back in 1993 in Paratuberculosis Newsletter (Chiodini, 1993) as an argument in support of the role of host factors in the pathogenesis of IBD. A recent paper by Stevenson's group reports low success in experimentally causing paratuberculosis lesions in sheep (Watkins et al., 2010). This is very similar to what more than 15 years ago a Spanish and a Mexican colleague (and probably many others) experienced to their despair when working on their PhD Thesis projects: MAP does not fulfil Koch's postulate that says that in vitro grown pathogens must cause disease (lesions) in susceptible animals. In fact, direct dosing with intestinal mycobacteria-containing extracts had become almost the standard for ovine experimental infections. Of course, an alternative explanation is that ruminant paratuberculosis is not really caused by MAP but by some other hidden factors that are usually present in the digestive system, including anomalous location of normal ruminal or intestinal flora.

Recent studies prove that the same genes in different species are involved in IBD pathogenesis (Pinedo et al., 2009a; Pinedo et al., 2009b; Ruiz-Larrañaga et al., 2010a; Ruiz-Larrañaga et al., 2010b). Since most of them are linked to innate immune response and the characteristic lesions also carry the landmark of primitive innate immune responses, which is macrophage activation and proliferation, this type of poorly known immune response is the best candidate for the development of a new pathogenesis model. This could prove difficult since we face at least three big challenges: a) the triggering external agent is often hidden, and their amounts are small, b) a specific immune response is not necessarily involved and if it is, it is as a collateral effect, and c) the threshold between infection and disease might be very fluid. I think Rod can successfully deal with these challenges and I hope these reflections can help.

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2. Events

The Japanese Society for Paratuberculosis is planning to hold a Specialized session for "Immunology of bovine paratuberculosis" supported by OECD and 9IVIS organizing committee in University of Tokyo.

The meeting will be held in August 16, 2010, as a satellite meeting to 9IVIS in Tokyo (http://9th-ivis.jtbcom.co.jp/).

Information about the specialized paratuberculosis session can be found at:
http://wwwsoc.nii.ac.jp/jsp3/pdf/OECDsymposium-E12SS.pdf

3. New Paratuberculosis Book

A new paratuberculosis book, **Paratuberculosis: Organism, Disease and Control**, was published recently. A number of IAP members have contributed to this book, edited by Marcel Behr and Des Collins. You may wish to have a look at the book:

http://bookshop.cabi.org/default.aspx?site=191&page=2633&pid=2215

Outline of the book

1. History of paratuberculosis
2. Global prevalence and economics of Infection with *Mycobacterium avium* subsp. *paratuberculosis* in ruminants
3. Epidemiology of paratuberculosis
4. *Mycobacterium avium* subsp. *paratuberculosis* in animal-derived foods and the environment
5. Paratuberculosis and Crohn's disease
6. Genetics of host susceptibility to paratuberculosis
7. *Mycobacterium avium* complex
8. *Mycobacterium avium* subsp. *paratuberculosis* genome
9. Molecular genetics of *Mycobacterium avium* subsp. *paratuberculosis*
10. Proteome and antigens of *Mycobacterium avium* subsp. *paratuberculosis*
11. Host-pathogen interactions and intracellular survival of *Mycobacterium avium* subsp. *paratuberculosis*
12. Comparative differences between strains of *Mycobacterium avium* subspecies *paratuberculosis*
13. *Mycobacterium avium* subsp. *paratuberculosis* and antimicrobial agents
14. Paratuberculosis in cattle
15. Paratuberculosis in sheep
16. Paratuberculosis in goats
17. Paratuberculosis in deer, camelids and other ruminants
18. Infection of non-ruminant wildlife by *Mycobacterium avium* subsp. *paratuberculosis*
19. Experimental ruminant models of paratuberculosis
20. Experimental small animal models of paratuberculosis
21. Immunology of paratuberculosis infection and disease
22. Cultivation of *Mycobacterium avium* subsp. *paratuberculosis*
23. Diagnosis of paratuberculosis by PCR
24. Immune-based diagnosis of paratuberculosis
25. Strain characterisation of *Mycobacterium avium* subsp. *paratuberculosis*
26. Paratuberculosis control measures in Europe
27. Paratuberculosis control in the USA
28. Paratuberculosis control measures in Australia
29. Ruminant aspects of paratuberculosis vaccination
30. Development of new paratuberculosis vaccines

4. List of Recent Publications

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