The Paratuberculosis Newsletter

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DEADLINE FOR NEXT ISSUE: May 15, 2011.

All contributions should be sent to ssn@life.ku.dk

Søren Saxmose Nielsen
Editor

1. IAP Business

Financial Report: Fourth Quarter 2010

International Association for Paratuberculosis

	Checking	Savings	CD	Total
Opening balance (1/1/10)	$15,921.81	$22,431.32	$56,221.76	$ 94,574.89
Q1 Closing balance (3/31/10)	$20,450.69	$18,068.48	$56,678.29	$ 95,197.46
Q2 Closing balance (6/30/10)	$21,801.21	$34,576.40	$57,104.41	$113,482.02
Q3 Closing balance (9/30/10)	$21,865.34	$33,544.93	$57,248.52	$112,658.79
Year End Balance (12/31/10)	$21,830.26	$33,553.38	$57,393.00	$112,776.64

Receipts

	Dues	Book Sales	Interest	10ICP	Total Receipts
Q1	$4700.00	$111.40	$459.25		$ 5,270.65
Q2	$1450.00	$ 14.25	$428.43	$16,568.00	$18,460.68
Q3	$ 100.00		$152.64		$ 252.64
Q4			$152.93		$ 152.03
Year Total	$6250.00	$125.65	$1193.25	$16,568.00	$24,136.90

Expenses

	Credit card processing fees	Webmaster	Total Expenses
Q1	$282.52	$4365.56 (Kennedy)	$ 4648.08
Q2	$176.12		$ 176.12
Q3	$ 35.87	$1040.00 (Banxui)	$ 1075.87
Q4	$ 35.08		$ 35.08
Year Total	$529.59	$5405.56	$5935.15

Net income

Q1	$ 622.57
Q2	$18,284.56
Q3	$ (823.23)
Q4	$ 117.85
Year Total:	$18,201.75

1. Short Scientific Reports

Mycobacterium avium subspecies *paratuberculosis* (MAP) infection in camel (*Camelus dromedaries*) in Saudi Arabia

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Summary

Dromedary camel (Camelus dromedarius) is one of the highly valuable domestic animals in Saudi Arabia. Camel is multipurpose animal that can be used for meat, milk and wool production. In addition to the previous traditional uses, modern applications in the dairy industry lead to the development of camel dairy farms that are capable of producing camel milk on the commercial level. Camel milk and meat are considered an important source of proteins for wide range of population. Recently, booming of camel racing in the Guelph countries made camel as highly attractive commodity (Breulmann et al., 2007).

In Saudi Arabia, Johne's disease was reported in sheep, goat, dairy cattle, and camel (Ahmed and Towfik, 1999, Gameel et al., 1994; Alluwaimi et al., 1999; Al Hajri and Alluwaimi, 2007, Alluwaimi, 2008, Al hebabi and Alluwaimi, 2010).

Although the general circumstantial evidence, samples from abattoirs, owner's observations and the veterinarian examination, indicated the severity of the MAP infection in camel, the detection of MAP infection in camel was hindered by efficient diagnostic tests. Hence, the efficiency of the commercial ruminant ELISA kit and the polymerase chain reaction (PCR) kits were examined for the detection of the camel Johne's disease. This approach was undertaken to provide a mean for effective national control plan and to disclose the overall picture of the disease in camel in Saudi Arabia.

Using bovine ELISA (ID VET, Montpellier, France), the pilot work on 100 samples from camel at different ages has revealed interesting and encouraging results (Alluwaimi, 2008). The study of camel MAP infection then pursued with wider application of commercial ELISA (Lsivet Ruminant Serum Paratuberculosis Screening Kit-France) and real-time PCR (VetAlert, Tetracore USA). The total of 861 serum and fecal samples were collected from the local abattoir which is the major provider of camel meat to the Eastern province of Saudi Arabia. The collected samples were categorized in three age groups, they were, the young age group (1-4 years old), the middle age group (5-9 years old) and the late age group (10-15 years old). Samples were distributed as follow 1-4 years old (276 samples) (71%), the 5-9 years old (23 samples) (8.5%) and 10-15 years old (11 samples) (5.5%).

The application of ELISA and PCR in this study has clearly indicated the influence of age susceptibility in camel to MAP infection. In addition, the retesting with the PCR reconfirmed the limitation of ELISA in detecting all animals that were exposed to the organism. The obtained results portrait the exact discrepancies of the MAP diagnosis in cattle using ELISA and PCR. Therefore, limitation of the PCR in revealing all shedding animals and the failure of the ELISA in detecting all seroconverted animals at the young age groups (groups 1-4 and 5-9 years old) portraits an embedded shortage in the ELISA and PCR as efficient tools for early detection of MAP infection. Hence, ELISA and PCR could play crucial role in monitoring the disease in camel but they are indecisive tools in providing clear picture on the extent of the MAP infection in the camel herds in Saudi Arabia. Nevertheless, they appear essential, especially ELISA, as the screening tests in the field.

The mechanism involved in the initiating the humoral immune responses were seen the major factors that render ELISA and PCR sensitivity variable at the early stage of the incubation period (Nielsen, 2008). Monitoring the shedding pattern of large numbers of cattle over 3 years illustrated five types of shedding patterns, non shedders, transient-shedders, intermittent-shedders, low shedders and high-shedders, on the basis of number of positive fecal culture and the detected MAP colonies. The results revealed fundamental findings regarding the relation of ELISA results to the pattern of shedding. The most intriguing findings were the possibility of using ELISA as forecasting tool for the commencement of shedding. In most cases, ELISA was capable of detecting seroconverted animals before the shedding commenced. However, some ELISA positive animals could remain fecal culture negative while others could start shedding before the seroconvertion (Nielsen, 2008). Hence, in view of these findings the results of ELISA and PCR in the camel MAP infection clearly reflect that PCR has unpredictable value due to the wide variations in the shedding pattern. However, the high numbers of positive ELISA in the age group 1-4 years old definitely support the predictive value of ELISA.

In view of these findings, a study was designed to reveal if the scenario of the shedding patterns is applicable in the camel MAP infection.

The results also reflected the cross reaction of MAP antigen in the ELISA kit with the camel anti-MAP antibodies and the sensitivity of the conjugated antibodies to detect camel's antibodies. The application of bovine ELISA for detecting anti-MAP in other species like deer was documented (Tryland et. al., 2004, Woodbury et. al., 2008).

Isolation of the camel MAP was also pursued in order to identify the relation of the camel strain to the other known MAP types. Unfortunately, repeated trails for isolation of the camel even for more than 3 months culture failed to reveal any detectable colonies. The possible growth of the organism was also failed to be detected by the PCR, however, the efforts are continued in this line.

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Paratuberculosis in cattle in Khartoum and Al-Jazeera States, Sudan; Clinicopathological and epizootiological studies

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Summary of MSc thesis

The high incidence of paratuberculosis (Johne's disease) in dairy farms was observed by veterinary investigators. Accordingly, this study was designed to investigate the clinicopathological and epizootiological aspects of the disease.

A total of 230 crossbred cattle between Friesian and Butana breeds, aged two years or older, from nine herds. Eight herds located at Khartoum State and one at Al-Jazeera State were surveyed for paratuberculosis. Twenty out of 228 (8.8%) animals were positive for faecal culture. Twenty three out of 225 (10.2%) animals were positive for ELISA test. Seventy three out of 230 (31.7%) animals were positive for acid fast bacilli (AFB) by faecal smear and twelve out of 86 (14%) animals were positive for rectal scraping.

Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in animals which had positive faecal culture in Khartoum State was 55.6% at the herd level and 8.8% at the individual animal level. Regarding sources, the lowest value of faecal culture positive (5.6%) was found at Soba and the highest (53.3%) at El-Sealate. Positive faecal culture was found in all locations with different prevalence except Wad-Medani. Khartoum North showed the highest faecal culture positive (13.2%) while Khartoum showed the lowest faecal culture positive (2.9%). There was positive correlation between faecal smear, rectal scraping and faecal culture and clinical signs.

The seroprevalence of paratuberculosis at Khartoum State was 66.7% at the herd level and 10.2% at the individual animal level. The lowest value of seroprevalence (8%) was found at kuku and the highest (18.8%) at El-Sealeat. All sera collected from El-Kadaro, Wad-Medani and El-Salama were found negative for MAP antibodies by ELISA test. Farms at Khartoum North showed the highest prevalence of seropositivity (12.7%) whereas Khartoum showed the lowest (7.1%). There was positive correlation between seroprevalence and clinical manifestations.

Four crossbred cows, 3-5 years old naturally infected with MAP were sacrificed and necropsied. Clinically, they showed profuse diarrhoea, emaciation and rough coat with area of alopecia on tail. The most prominent gross lesions were thickening, oedema and corrugation of the wall of small and large intestine. The mesenteric lymph nodes were swollen, oedematous. Histopathologically, all animals presented granulomatous enteritis. The inflammatory exudates varied from accumulation of lymphoid cells mixed with some epithelioid macrophages and giant cells to sheet of epithelioid macrophages intermingled with some lymphoid cells. The lymphatic vessels in submucosa of both small and large intestine were dilatated and filled with pink homogenous proteinous materials. Acid fast bacilli were demonstrated in infiltrating epithelioid macrophages and giant cells. The sera of the four animals were positive for MAP antibodies by ELISA test. Inocula prepared from small and large intestine and mesenteric lymph nodes of the four animals showed small, round, smooth and glistening colonies on the 5-7 weeks incubation.

2. Comments & Opinions

I'm Back Ramon

Rod Chiodini

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Authors Preface: For those members not familiar with us "old folks", appreciate that Ramon and I are friends and have shared many B33Rs together, probably too many. Anything said in this article that may sound antagonistic, probably is, but it is done in fun and in the spirit of our long standing friendship and mutual intellectual prodding.

In the June 2010 issue of The Paratuberculosis Newsletter, Ramon Juste, President of the Association, wrote an article in the Comments & Opinions section entitled "Welcome Back Rod!"[1]. For those of you who did not read that article and/or have forgotten about it, his article was in reply to something I wrote relating to establishing a causal link between *M. paratuberculosis* and Crohn's disease. I have no idea what I wrote back then (us old folks tend to forget things), but that doesn't really matter.

Before I tell Ramon why he is full of s%@&#t, let me make my position clear.

1. I do not believe in Crohn's Disease. I believe in Crohn's diseases or a Crohn's disease syndrome, i.e., many diseases of different etiologies grouped into what is commonly called Crohn's disease. As long as we treat Crohn's disease as a single disease entity we will never determine the cause anymore than we would make any advances in cancer if we treated all cancer the same.

2. I do not believe that *M. paratuberculosis* causes Crohn's disease. Rather, I believe that some cases diagnosed as Crohn's disease are caused by *M. paratuberculosis*. For reasons I will not go into here, I believe that it is 10-30% of cases.

Also, appreciate that I am very simple minded. I find that most things, when they are understood, are generally simple. I also find that when things do not make sense, it is not generally related to our lack of understanding of the complexities, but rather, they simply do not make sense, i.e., something is wrong. This will have relevance later.

In Ramon's "rebuttal" to my article, he keeps on referring to Koch's postulates as if that were my basis of thought, which it is not. I challenge Ramon to show a single reference to any article that I have ever written where I refer to Koch's postulates. Besides Ramon, you misinterpret Koch's postulates – they were broad principles (not rules) which, when dealing with infectious agents, can be broadly applied to most known infectious diseases (where the lack of species specify allows it to be applied). As Ramon noted, when Koch proposed his postulates, he himself recognized that they were not fully applicable, nor could they be strictly applied, to all infectious agents and hence they were general principles. That being said, let's move on….

Most other comments made by Ramon can be discounted simply because he uses negative projections or speculations to dismiss or refute them. To question a

proposed experiment because it "might fail" is not solid ground on which to dismiss it. While something "might fail", it also is possible that it "might succeed" and you will never know until you try. Isn't that the basis of scientific inquiry? You develop a hypothesis and then design experiments to support that hypothesis. The results may fail or they may succeed. But all this is trivia …

I disagree with Ramon that "brilliant scientists using relatively large resources and energies" have failed and therefore we shouldn't keep trying. There have been few truly comprehensive studies using new or "old" methodologies and approaches that do not leave unanswered questions or, more frequently, raise additional unanswered questions. You cannot continue to do basically the same thing (search for IS900 for example) and expect to get different results. Classical approaches, using refined methodologies, may be appropriate to define/redefine critical issues in the debate. As Ramon noted, the simple and classical approach may fail. But so might the complex and in my simple mindedness, I prefer the simple before jumping into the complex.

There is little doubt that Crohn's disease is a complex disease that is influenced by host susceptibility (genetics), environmental, and immunologic factors. But then again, can't we say the same thing and develop this level of complexity with all diseases? If an infectious agent is involved (*M. paratuberculosis* or other), then the agents genetics (virulence) also come into play.

With that being said, most are open to the notion that some unique concepts and disease models (even lactose intolerance[2] if you like) will likely apply, but there is a limit to what can be reasonably accepted. One cannot come forth with half a dozen new concepts that do not have any comparative model and expect to receive broad acceptance. This is particularly true when these new concepts are used to explain inexplicable research results. At that point, they become excuses rather than hypotheses.

There are really 2 major concepts in the *M. paratuberculosis*-Crohn's disease debate in which all others can basically be grouped.

1. Progressive disease results from very small numbers of organisms. There is no other comparative disease. Tuberculoid leprosy is not progressive (in the paucibacillary state) and is self-limiting[3]. It is questionable whether paucibacillary paratuberculosis is truly progressive leading to clinical disease and not simply reflecting early disease lesions[4] (and in alleged clinical paucibacillary cases, a thorough workup to rule out other factors that could have caused clinical disease are not generally ruled out; i.e., the paucibacillary lesions could be incidental to clinical signs). It is a novel concept that low bacillary loads can result in progressive disease without ever progressing to a multibacillary disease, even in the presence of immunosuppressive and immunomodulating agents. By the same token, there is a big difference between "difficult to find" and not being able to find.

2. We are all exposed to *M. paratuberculosis* as an environmental organism, but Crohn's disease patients are uniquely susceptible. There is no other chronic progressive disease caused by an environmental organism in non-immunocompromised individuals. Patients with Crohn's disease are not immunocompromised (other than therapeutically) and are not susceptible to other opportunistic infections. This would have to be an extremely unique susceptibility (genetic or otherwise) specific to *M. paratuberculosis* and not other mycobacteria

(environmental or otherwise) or other opportunistic organisms. I find this novel concept to be more difficult to swallow than #1 above which brings me to my main point, my main question(s), and my main concern(s):

Is *M. paratuberculosis* an environmental organism? and **What is *M. paratuberculosis*?**

As I will elucidate below, these two questions are closely inter-related. It is the answer to these questions that may have the greatest impact on our understanding of the data generated on *M. paratuberculosis* as it relates to Crohn's disease and developing hypotheses based on solid scientifically sound background data. It is the answer to these questions that could have a major impact on the development of rational disease prevention and management programs in both human and veterinary medicine and deal with the enormous challenges in the control of Johne's disease.

It is these two questions that I would most like to hear comments on from Ramon and other experts in the field. It is these two questions that I hope to grab your attention and rattle a few cages at the very least.

According to recent publications, *M. paratuberculosis* is widely distributed in the environment and has been (can be) detected in a host of food products[5], rivers, streams, lakes[6], and even in 81% of tap water samples.[7] These data suggest that we are all exposed to *M. paratuberculosis* on a near daily basis which, by definition and default, would classify *M. paratuberculosis* as an environmental opportunistic organism. This would also mean that the main biomass of *M. paratuberculosis* is the environment and not infected domestic livestock as we have always been lead to believe. If we accept this fact, that the main biomass of *M. paratuberculosis* is the environment, we must then ask the question why all domestic and wild ruminants are not infected?

It is this exact question and observation that lead to changes in the taxonomy of the *M. avium* complex.[8] If *M. avium* subsp. *avium* (the causative agent of avian tuberculosis) is a widely distributed environmental organism, why are not all birds infected? Lo and behold, further investigations into this question revealed that *M. avium* subsp. *avium* was not an environmental organism after all (which is why all birds are not infected). The environmental strain was found to be the apparent original ancestor of the *M. avium* complex, now known as *M. avium* subsp. *hominissuis*.[8]

Therefore, we must come to grips with the question of whether or not *M. paratuberculosis* is an environmental organism. If it is, we must ask ourselves these 2 simple questions (remember, I am simple minded):

1. Why are not all ruminants infected since they are apparently exposed to this infectious agent on a daily basis? We may need to completely rethink our entire thought pattern related to the pathogenesis of Johne's disease as it is no longer a classical bacterial infection but rather a complex disease involving a host of unique environmental, genetic, immunologic, and virulence factors, and an *opportunistic pathogen*.

2. Is *M. paratuberculosis* really the cause of Johne's disease? Maybe Ramon was not joking when he said "*an alternate explanation is that ruminant paratuberculosis is not really caused by MAP*…"[1]. Perhaps *M. paratuberculosis* is similar to the leprosy-associated corynebactera[9] and really has nothing to do with Johne's disease?

I personally don't buy either of those explanations. While it is possible that we have been completely wrong for 100+ years about Johne's disease and *M. paratuberculosis*,[10] I find that a hard pill to swallow. However, on the positive side, we may have just created a comparative animal model for the development of a specific progressive disease caused by an environmental organism in non-immunocompromised hosts.

We either have to accept and face the challenges above, or conclude that *M. paratuberculosis* is not an environmental organism. If we accept that fact, we are now faced with explaining the detection of *M. paratuberculosis* in all these environmental sources, which brings me to the second question: What is *M. paratuberculosis*?

Historically, for almost 100-years, *M. paratuberculosis* has been identified as acid-fast, slow growth (12-16 weeks), and mycobactin dependent.[10] While these characteristics could be arguably rudimentary, they have proven themselves to be reliable within the context of their use, I.e., identification of *M. paratuberculosis* in ruminant animals. With the advent of molecular biology and identification of IS900, this addition criterion added some precision and objectivity to the identification of *M. paratuberculosis*.

However, 2 very important things have happened in recent years that must raise questions or at the very least, some concern:

1. The detection of IS900 has become the absolute unequivocal evidence of *M. paratuberculosis* to the exclusion of all else. While this may be appropriate (i.e., IS900 is *prima fascia* evidence of *M. paratuberculosis*), some recent conflicting data raises some concerns.

2. We have applied workable methodologies (IS900) within defined ecosystems (ruminant populations) to exogenous environments without validating these methodologies within these new ecosystems.

We have become almost completely reliant on the identification of *M. paratuberculosis* based on the detection of IS900. Slow growth and mycobactin dependency have become almost obsolete, particularly when dealing with newer liquid culture methodologies and approaches.[11] Acid-fastness may not even be a characteristic anymore with more and more publications suggesting non-acid-fast pleomorphic material in culture media identified as *M. paratuberculosis* based on IS900 detection.[12] As I will highlight below, if you look at available published data, all characteristics of *M. paratuberculosis* have been found to be obsolete and not even necessary for identification, which makes me wonder what *M. paratuberculosis* really is?

Within domestic livestock populations, the use of IS900 has been time-proven to be specific, sensitive, and reliable and the detection of IS900 can be directly correlated

with *M. paratuberculosis* with a reasonable degree to certainty. However, does this specificity and reliability hold true in other ecosystems?

We generally define species specificity based on the lack of homology with known genomic sequences in other species (i.e., genomic databases) and the examination of similar and dissimilar isolates.[13] In most cases, these examinations have been with various bacterial strains and species of animal origin thereby validating species-specificity within the ruminant ecosystem. But is this adequate without further validation when our comparisons reflect only a fraction of species within the ecosystem and a fraction of the genomic diversity of life? Is it sufficient validation for use in remote ecosystems (like in humans) without examination of bacterial strains and species associated with that ecosystem?

In the human intestinal tract, there are approximately 1500 different bacterial species that make up the human intestinal microbiome, with each individual containing 150-160 unique species.[14] Of those 150-160 species, about 60% are common (i.e., shared amongst all humans) and about 40% are unique to that individual. What is most interesting is that 80% of all bacteria in human feces are unknown and have never been cultured. Sequencing of the bacterial 16s ribosomal genes in feces produce sequences (80%) that cannot be aligned with any known species of bacteria (and hence bacteria that have never been cultured). [14] With 100 trillion bacteria in the intestine (containing >100 times more unique genes than in the entire human genome), of which 80% are uncultivable and unknown, can we confidently declare species-specificity without further validation?

Knowing that we are basing our designations (species-specificity) on a small fraction of the total bacterial populations in any given ecosystem, let's go back and look at what we call *M. paratuberculosis*. As stated earlier, *M. paratuberculosis* is a slow-growing (12-16 weeks) acid-fast bacterial species that is mycobactin-dependent and contains the insertion sequence IS900. I think most of us can agree that this comprises our full definition and identification criteria for *M. paratuberculosis*.

With improved culture techniques, particularly liquid culture, we have redefined the growth rate such that the original 12-16 week growth requirement has become largely obsolete.[5] Liquid culture methods also circumvent the mycobactin-dependency requirement since *M. paratuberculosis* has long been known to be capable of growth (albeit slower) in liquid cultures without mycobactin.[15] Furthermore, stains identified as *M. paratuberculosis* have been found that are described as not requiring mycobactin.[8] Is this even possible?

According to the genomic sequence of *M. paratuberculosis*, the mycobactin synthesis operon is truncated,[16] which likely accounts for its inability to produce mycobactin and hence its dependence on exogenous sources. For *M. paratuberculosis* to lose its mycobactin dependency, it would require this gene to be "repaired", a novel situation and concept in bacterial genetics. The only other explanations would be that these strains represent ancestral organisms of *M. paratuberculosis* prior to the loss of mycobactin-dependency (like *M. avium* subsp. *hominissuis*) or they are not *M. paratuberculosis*. Ramon, can you come up with any other rational explanation(s)?

This enigma is perhaps best illustrated in the publication by Naser *et al* where 73% of *M. avium* strains isolated from patients with AIDS were found to contain IS900.[17] These strains, originally grown in human diagnostic laboratories that do not incorporate mycobactin in their media nor wait 12-16 weeks for growth, illustrates that slow-growth and mycobactin-dependence do not necessarily correlate with the presence of IS900. It should also be noted that the primers used in this study were P90/P91; the most commonly used "species-specific" IS900 primers. There are only 2 possible explanations for these findings: 1) that there are strains of *M. paratuberculosis* that are not slow-growing or mycobactin-dependent that are commonly infecting immunocompromised patients or 2) that some other yet to be identified organism(s) also contain IS900. Looking for the simple answer, I must lean towards the latter; i.e., IS900 may not be species-specific for *M. paratuberculosis*.

And what do we make of those strains of *M. paratuberculosis* that are claimed not to contain IS900?[18] I am now totally confused!

Within our limited knowledge base (20% of the bacteria in any given ecosystem), we have found IS900-like sequences in other species, such as *M. porcinum* (which is positive with the commonly used P90/P91 primer set).[19] Is it a large leap of faith to suggest that sequences identical to IS900 may be present in some species that comprise the 80% of unknown bacteria within the microbiome of distinct ecosystems?

Adding fuel to the unknown is the fact that "species-specific" insertion sequences have been shown to cross species barriers.[20] If IS1245, which is species-specific for *M. avium*, can undergo natural translocation into *M. kansasii*, is IS1245 not really species-specific for *M. avium* after all or does *M. kansasii* now become *M. avium*? Aren't insertion sequences also known as transposable elements and jumping genes? Would it be that surprising to find the same natural translocation with IS900?

Considering the facts and enigmas above, can you with confidence identify an organism as *M. paratuberculosis* based on the presence of IS900 alone, particularly outside the ruminant ecosystem? If you can't, what do you make of all these reports that identify *M. paratuberculosis* based on the presence of IS900 alone? Would not the presence of IS900 in some yet to be identified microorganism explain everything?

It would explain the widespread environmental distribution of IS900 ("*M. paratuberculosis*") without widespread disease. It would explain the widespread detection of IS900 ("*M. paratuberculosis*") in normal human blood and tissues. It would even explain the difficulty in some experiments to infect animals with IS900-positive strains referred to by Ramon.[1] It would actually explain every enigma and conflicting data as related to both Johne's disease and Crohn's disease. I am open to other simple explanations.

Again let me stress that the presence of IS900 has proven itself as a confirmatory test for the detection and identification of *M. paratuberculosis* in ruminant livestock populations. My questions relate to the application of IS900 detection as the sole basis for the identification of *M. paratuberculosis* in remote ecosystems such as the environment and human populations.

And this all brings me back to my original question, what is *M. paratuberculosis*? It may or may not be acid-fast. It may or may not be slow-growing. It may or may not be mycobactin-dependent. And it may or may not even contain IS900 in some circumstances. Do we really know the characteristics of *M. paratuberculosis* anymore?

I think we need to take a step back and take a good hard look at what we are calling *M. paratuberculosis,* particularly when we are outside the "comfort zone" of the domestic livestock ecosystem.

I think we need to look at these various strains "isolated" from remote ecosystems by 16s sequencing and compare those to classical *M. paratuberculosis* from infected ruminants. Has there even been sufficient sequencing of *M. paratuberculosis* 16s ribosomal DNA to have a clear and concise definition of the species as compared to *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*? Do these IS900+ strains that are not mycobactin dependent and not slow growing (such as those from AIDS patients) type to *M. paratuberculosis* or do they type to another *M. avium* subspecies or even another *Mycobacterium* containing IS900? How about all these alleged spheroplasts "isolated" in liquid cultures from normal blood, river and tap water, etc? Are they pure cultures and do they actually type to a *bona fide M. paratuberculosis* strain based on 16s sequencing? What are we calling *M. paratuberculosis*?

I am convinced that, although all *M. paratuberculosis* strains are IS900-positive, the presence of IS900 alone may not be sufficient to claim something is *M. paratuberculosis*.

And why am I bringing all this up and how does this all relate to the original issue of *M. paratuberculosis* and Crohn's disease and establishing causality? Simply because any theory related to *M. paratuberculosis* and Crohn's disease, from the simple to the complex, will have little meaning until we define exactly what we are detecting with IS900, determine if *M. paratuberculosis* is or is not an environmental organism, and explain the enigmas created by those determinations.

Now, it is possible that I could be completely wrong and that all of the above could just by my idiotic senseless ranting. After all, I think I was wrong once before (or was that the time I just thought I was wrong?)

But, if there are others out there that agree that we have a problem that really needs to be sorted out to make sense of all this conflicting data and are interested in sorting out these enigmas, I might be willing to collaborate and do the 16s sequencing.

If nobody is interested, will somebody at least please tell me what *M. paratuberculosis* is as I am completely confused? Have I rattled any cages? Have I gotten you to at least think and consider that things are not making sense?

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Are *Mycobacterium avium* subspecies *avium* and *Mycobacterium avium* Complex Pathogens?

Gilles R. G. Monif, M.D.

Current diagnostic tests focused on identifying pathogen mycobacteria that cause granulomatous enteritis in herbivores have largely dismissed *Mycobacterium avium* subspecies *avium* (Maa) and *Mycobacterium avium* complex (Mac) as being environmental organisms. Like *Mycobacterium avium* subspecies *paratuberculosis* (Map), Maa is embedded in the environment. Maa can be isolated from water, dust, and soil. Having established the "gold standard" for the documentation of Johne's disease as being isolation of *Mycobacterium avium* subspecies *paratuberculosis*, the need arose to confer specificity to the diagnosis. The decision was made to dismiss recovery of *Mycobacterium avium* subspecies *avium* and *Mycobacterium avium* complex (Maa/Mac) isolates as being environmental contaminates in fecal cultures, rather than assessing their possible pathogenicity in a given case. Given that the vast preponderance of cases of Johne's disease is due to Map, these deletions from diagnostic analysis served a pragmatic solution. The negative residue of this decision has been to understate disease due to Maa/Mac in domestic animals.

Maa/Mac disease in humans

Maa/Mac are among the predominant causes of disseminated mycobacteremia in individuals with AIDS (1). Environmental, not human-to-human, transmission is the primary source of infection, with the primary portal of entry being the gastrointestinal tract (2). Hellyer et al. have document gastrointestinal involvement and inferred that it is the portal of infection for Maa (3.) Using an animal model system, Bermudez et al. demonstrated *Mycobacterium avium* complex disseminated infection after colonization of the gastrointestinal tract (4). Exposure to low oxygen tension and increased osmolarity that occurs in the gastrointestinal tract has been demonstrated to enhance the ability of Mycobacterium avium to enter epithelial cells. Yoder et al. recovered mycobacterium from 25 of 121 food samples tested. Of the 12 Maa isolates, a relationship between food isolates and clinical patient isolates was demons ratable in three cases (5).

Characteristically, granulomatous enteritis due to Maa/Mac in humans occurs primarily in severely immunocompromised individuals. This presumed pre-requisite condition compromised the ability of the veterinary world to seriously entertain that Maa/Mac could cause granulomatous enteritis (Johne's disease) in immunocompetent cattle. The validity of that line of reasoning is undermined by the fact that Mac disease can occur in non-immunocompromised humans (6, 7).

Maa/Mac in Animals

Clinical studies have shown that Maa/Mac have been recovered from diseased and non-diseased birds, ducks, rabbits, squirrels, cats, dogs, swine, horses, free-ranging deer, and elk (8-25). Maa/Mac is a significant mycobacterium pathogen in pigs and horses. Despite Maa/Mac being occasional recovery from cheese, infection of dairy cows had presumed not to occur.

Does Maa/Mac cause infection/disease in cows?

Mathews and McDiarmid produced in cows a disease resembling paratuberculosis with a mycobacterium isolated from a woodpigeon (26). Williams et al. indirectly looked at the issue of the potential bovine pathogenicity of Maa (27). Three hundred and sixty eight fecal samples from dairy cows enrolled in USDA's Florida Johne's Disease Demonstration Project were analyzed in two independent laboratories. The fecal samples delivered to Purdue University School of Medicine were tested using both culture and heat shock protein gene (Hsp X)-based Map real-time PCR testing. The portion delivered to the Diagnostic Laboratory at the University of Florida College of Veterinary Medicine was analyzed using a nested IS*1311*-based PCR test. The nested IS*1311* PCR test identifies both mycobacterium in the Mac grouping as well as Map. One out of the 368 fecal specimens analyzed by all three methods grew out heavy 4+ growth of a mycobacterium not identified as Map by IS*900* primers. The corresponding heat shock protein analysis was also strongly positive as were the direct and nested IS*1311* PCR (FecaMap®. The animal was culled shortly thereafter. Not being identified as Map, the isolate was discarded before a definitive identity could be established. Of the 85 mycobacterium isolates available through USDA, 5 Maa isolates, 7 *M. intracellulare*, and 10 *M. hominisusuis* were identified as coming from cows. Of the five Maa isolates and 7 *M. hominissuis* isolates, 4 (80%) and 5 (50%) respectively share Map02 confirmation with Map (28).

What the literature has documented is that Maa/Mac organisms are potentially pathogenic mycobacteria that attain host access through the gastrointestinal tract, and that cause chronic granulomatous enteritis.

Confirmation of possible pathogenic mycobacterium clinical isolates cannot be simply done using IS*900* primers. One needs to first test with IS*1311* PCR primers and then refine the specificity with IS*900* PCR primers. Otherwise, the true prevalence of pathogenic mycobacterium in bovine herds will continued to be underestimated.

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3. Events

11th International Colloquium on Paratuberculosis 2012

We warmly invite you to join us for

The 11th International Colloquium on Paratuberculosis 2012

5-10 February 2012 | Sydney | Australia

Cosmopolitan Sydney is Australia's largest and most exciting city, the perfect destination for a colloquium as significant as this. Sydney is one of the world's most beautiful cities, known for its famous harbour, beaches and national parks and boasts a stunning location, temperate climate, world-leading facilities and infrastructure, a robust economy and friendly locals. Sydney is simply unforgettable.

During this five day colloquium we will bring together a vast array of international expertise in an exciting scientific program including presentations from the following fields:

The Scientific Program highlights Include:

- 3rd ParaTB Forum (by invitation)
- Diagnostics and detection of MAP
- Host response and immunology
- Control Programs
- Pathogenomics
- Mycobacterial diseases of wildlife
- Genotyping and MAP diversity
- Industry forum
- Epidemiology
- Public Health and MAP in the environment
- International initiatives
- Synopsis and future directions

There are plenty of opportunities to catch up with old friends, meet new ones whilst enjoying the beauty of Sydney and it's surrounds during a Welcome reception, **Harbour cruise**, **Taronga Zoo** excursion and the highlight **Colloquium dinner.**

We are delighted to announce the following speakers;

Ian Gardner

Before becoming the Canada Excellence Research Chair in Aquatic Epidemiology, Dr. Ian A. Gardner was professor of medicine and epidemiology at the School of Veterinary Medicine, University of California, Davis. Ian earned his bachelor's degree in veterinary science from the University of Sydney, and worked in his native Australia as a veterinary officer specializing in pig and poultry diseases.

Professor Gardner is internationally recognized for developing methods for validation of diagnostic tests for animal diseases and to assess disease risk in terrestrial and aquatic food animals. These methods have been used in global veterinary and public health activities, and have influenced policies at the United States Department of Agriculture and internationally through the World Organization for Animal Health.

Professor Gardner is among the most cited researchers in his field, with more than 200 peer-reviewed scientific publications in leading journals, such as *Preventive Veterinary Medicine*, *Journal of the American Veterinary Medical Association*, and *Veterinary Pathology*.

Jayne Hope

Dr Hope is group leader focusing on research into innate and adaptive immune mechanisms in cattle at the Institute for Animal Health, UK. Her group is carrying out research into bovine tuberculosis (TB) with the aim of understanding immune mechanisms that lead to protective immunity in mycobacterial disease.

Dr Hope obtained a BSc (Hons) in Biological Sciences (Microbiology) from the University of Birmingham, UK in 1991, and a PhD from the University of Manchester, UK, in 1994. She carried out postdoctoral research at the University of Manchester (1994-1996) and Kings College School of Medicine and Dentistry (1996-1997), before joining the Institute of Animal Health to research major chronic diseases of livestock.

Eiichi Momotani

Dr Eiichi Momotani (DVM, PhD), lives in Tsukuba, Japan. He is the Senior Researcher for the National Institute of Animal Health of Japan with a focus on immuno-pathology, molecular biology and proteomics, an affiliate professor at Azabu University and an Adjunct Professor in Ibaraki Prefectural University of Health Sciences. His most cited work in paratuberculosis was the first observation of the interaction of MAP with M cells, and his most recent work centres on mouse models to explore possible links of MAP with Crohn's disease.

He has previously been an Adjunct Professor in Microbiology at the Faculty of Medicine in Hokkaido University, Japan as well as holding similar roles in Ibaraki Prefectural University of Health Sciences, and the University of Tokyo. Dr Momotani serves as a delegate on Government committees, is a journal reviewer, and has received several awards for his work. He is the author of many professional articles and 7 books on veterinary science and is a most engaging speaker.

Expressions of Interest:

Further information regarding the program, awards and abstracts will soon be announced, however in the meantime, please visit the website to register your expression of interest in attending and being a part of the International Colloquium on Paratuberculosis 2012.

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4. List of Recent Publications

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