Message from the President

Join me for good science and great friends in Copenhagen this August.

Mike Collins, President, IAP
Notes from the editor

After 10 years as Editor in Chief of the Paratuberculosis Newsletter it is more than time to step down and to pass on the job to a younger, more active mind. The inclusion of information from my PhD thesis written way back in last century highlights this need. Claus Buergelt sent me some information for inclusion in the Newsletter on the isolation of *M. paratuberculosis* from the blood stream of a cow with Johne’s disease. His observation of finding *M. paratuberculosis* in the blood stream has a number of important consequences. Firstly, it re-emphasises the fact that major lesions are only present in the gut and its associated lymph nodes, even though the organism is present in the blood stream. The reason for this tissue tropism still remains to be elucidated. Importantly, the finding of the organism in the blood stream highlights the need to consider meat as a possible source of *M. paratuberculosis* for humans. If *M. paratuberculosis* is present in the blood, it almost certainly will also be present in muscle. The implications of this observation will be major if a causal link between *M. paratuberculosis* and Crohn’s disease is demonstrated. If food safety authorities are following a precautionary approach they have a significant challenge when developing protocols to reduce the level of *M. paratuberculosis* in food. The recent paper by Pickup and colleagues identifies water as another potential source of infection (See “Editor’s Selection” for the abstract of this paper).

I would like to thank the people who have contributed to the Newsletter over the years. Without your contributions the task would have been all that more difficult. I would especially like to thank Mike Collins for his support and encouragement over the years. He has put a lot of time and energy into the International Association for Paratuberculosis something for which we should all be very grateful. Recently Mike in an unwise moment to raise my spirits sent me some pictures of his adventures in Chile. The one below appears to indicate that he is coping very well with the stresses of being President of our organisation.
To those members who have not contributed to the Newsletter I would urge them to do so. I am sure that lots of people have information they could contribute to the Newsletter. All that is required is to sit in front of a computer for a little while and then press “send”. In many cases prepared information that has had a limited circulation in your home country will be of interest to the wider Paratuberculosis community.

Geoffrey W. de Lisle
Editor

Geoffrey.delisleg@agresearch.co.nz AgResearch, Wallaceville Animal Research Centre, P.O. Box 40-063, Upper Hutt, New Zealand
Communication

Detection of viable Mycobacterium avium subsp. paratuberculosis by Culture in the Blood of a Dairy Cow with Johne’s Disease.

Claus D. Buergelt and J. Elliot Williams

University of Florida

Background

Johne’s disease, a chronic infectious disease caused by Mycobacterium avium subsp. paratuberculosis (Map), affects domesticated, wild and zoo ruminants. The intestinal tract and associated draining mesenteric lymph nodes are the target site for the disease to develop. The organism can be cultured from feces. It has been established in the literature that Map can be successfully isolated by culture from milk, semen and fetuses of infected cows suggesting an extra-intestinal site for survival. Pathologic alterations as seen in the intestinal tract and associated lymph nodes are absent in these distant organs. It is postulated that as a facultative intracellular organism, Map reaches these sites through circulating monocytes.

A recently developed nested polymerase chain reaction (nPCR) in our laboratory has utilized peripheral blood monocytes obtained by gradient centrifugation for the diagnosis of clinical Johne’s disease and subclinical infection. To the knowledge of the authors cultural isolation of Map from bovine blood has not been previously reported meaning that detection of viable Map in blood is suggestive of systemic bacteremia. This communication describes the successful isolation of Map from the blood of a dairy cow exhibiting clinical signs of Johne’s disease. The detection coincides with a recent publication of culture of viable Map from the blood of human patients with Crohn’s disease.

Case Report

A 2.5 year old Holstein dairy cow from a Johne’s disease proven herd developed clinical signs of diarrhea, weight loss and milk reduction shortly after calving. The cow was subjected to serologic analysis of Johne’s disease through ELISA and AGID testing. A nPCR was performed on peripheral blood and milk samples. The animal had a strong positive ELISA titer and positive bands on AGID. The nPCR resulted in a positive amplicon for Map DNA on peripheral blood monocytes, but was negative on the milk sample. With a confirmative clinical diagnosis of Johne’s disease the animal was euthanized and submitted for a necropsy. Necropsy provided the pathologic diagnosis of a granulomatous enteritis and lymphadenitis. Multiple acid-fast bacilli were demonstrated with special stains confirming the case as multibacillary Johne’s disease.
Blood monocytes collected before euthanasia and prepared from 10 ml of EDTA containing tubes by gradient centrifugation were subjected to slants containing Herrold’s egg yolk agar with and without mycobactin. After 2 months of incubation at 37°C a single small white colony was detected on one slant containing mycobactin. The colony was positive with an acid-fast stain. Typically bacilli were arranged in clumps. (Fig. 1). When subjected to nPCR, amplicons were visualized on gel electrophoresis at the expected locations with primers for simple PCR and with primers for nPCR. (Fig.2)

Conclusion

The isolation of Map from peripheral blood monocytes, and the biochemical and molecular characterizations of Map add credence to a bacteremic phase and hematological spread of the organisms to extraintestinal sites. It supports the emphasis that Map infection is disseminated and systemic and needs to be differentiated from the disease phase which has the intestinal tract as target site. The detection of viable Map in blood supports the hypothesis that dissemination of Map is actively achieved thorough circulating monocytes. Our findings should invite others to duplicate and verify our observations. A larger volume of blood might increase the likelihood of obtaining more positive peripheral blood cultures from individual animals.

Finally, the relative young age of this animal with clinical signs of Johne’s disease might be a reflection of a possible in-utero transmission of *Mycobacterium avium* subsp. *paratuberculosis*.

Selected References


Legends

Figure 1. Smear from slant colony stained for acid-fastness. Notice individual and clumped bacilli. Fite’s acid-fast, x100.

Figure 2. Gel electrophoresis of IS900 amplification products of blood culture positive organisms. Single bands at location between 400 and 300bp. Simple PCR lanes 1-3 using primers P90,P91; nPCR lanes 5-10 using primers J1,J2.
Lane 1: positive control from lab strain #295; lane 2 reaction product of blood monocytes with first set of P90,P91 primers; lane 3 dH2O as negative control; lane 4 molecular size markers (Promega); lane 5 positive control from lab strain #295 from simple PCR; lane 6 positive control from lab strain #295 with nPCR, lane 7 reaction product of blood monocytes with second set of J1,J2 primers; lane 8 negative control with dH2O from simple PCR, lane 9 negative control with dH2O from nPCR; lane 10 molecular size markers (Promega)
The isolation of *Mycobacterium paratuberculosis* from blood of cattle

G.W. de Lisle

AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand

The following are extracts from my PHD thesis (1979).

**Part 1. Bacteriology, Materials and Methods:**

**Experiment 3. (a) Buffer coat cultures.**
Buffy coats were cultured from 6 animals which were persistent shedders and the results are summarised in Table 1.5. No decontamination step was included in the procedure as a check against possible contamination from sources outside the blood stream. Some of the initial contamination was probably due to the use of non-sterile heparinized vacutainers. A switch to sterile heparin reduced the amount of contamination. Isolates were obtained in the absence of contaminants which would indicate they were obtained from peripheral blood.

**Results.**

**Table 1.5. Blood cultures from persistently shedding cattle**

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>No. samples cultured</th>
<th>No. contaminated*</th>
<th>No. culture positive**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corine</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>158</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Smokey</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Jill</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gibson</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>M69</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* All four tubes of Herrold’s egg yolk medium contaminated for each sample.

**Discussion**

“The isolation of *Mycobacterium paratuberculosis* from buffy coat cells indicates that animals with advanced Johne’s disease have a low grade bacteremia. One would hypothesize that the bacilli are contained within circulating monocytes. The isolation procedure did not exclude the possibility of bacteria also in the plasma. Alexejeff (1929) reported the isolation of *M. paratuberculosis* from the blood but the isolation procedure was not described. Goudswaard (1971) isolated *M. paratuberculosis* from the blood of heavily infected goats. The isolation of *M. paratuberculosis* from organs not associated with the intestinal tract, such as the kidney and bronchial lymph node, is further evidence of a bacteremia. The failure to demonstrate histopathological
lesions (A.H. Corner and J.R. Duncan pers. com.) in extra-intestinal organs is evidence against the multiplication of *M. paratuberculosis* in these organs. Lesions have been observed in the liver, hepatic lymph node and tonsil, however these organs are classed as gut associated tissues. Only small numbers of bacilli were isolated from extra-intestinal organs. These results are suggestive that the specific conditions for the multiplication of *M. paratuberculosis* are only present in the intestinal tract and associated tissues.”

*Editors Comments. There can be little doubt that if *M. paratuberculosis* is present in the blood stream of heavily infected cattle it will also be present in muscle. Whether this is an important source of infection for humans is unknown.*

**Reference**


The Editor’s Selection

Mycobacterium avium subsp. paratuberculosis in the Catchment Area and Water of the River Taff in South Wales, United Kingdom, and Its Potential Relationship to Clustering of Crohn's Disease Cases in the City of Cardiff.


Centre for Ecology and Hydrology, Library Ave., Bailrigg, Lancaster LA21 4AP, United Kingdom. roger@ceh.ac.uk.


In South Wales, United Kingdom, a populated coastal region lies beneath hill pastures grazed by livestock in which Mycobacterium avium subsp. paratuberculosis is endemic. The Taff is a spate river running off the hills and through the principal city of Cardiff. We sampled Taff water above Cardiff twice weekly from November 2001 to November 2002. M. avium subsp. paratuberculosis was detected by IS900 PCR and culture. Thirty-one of 96 daily samples (32.3%) were IS900 PCR positive, and 12 grew M. avium subsp. paratuberculosis bovine strains. Amplicon sequences from colonies were identical to the sequence with GenBank accession no. X16293, whereas 16 of 19 sequences from river water DNA extracts had a single-nucleotide polymorphism at position 214. This is consistent with a different strain of M. avium subsp. paratuberculosis in the river, which is unculturable by the methods we used. Parallel studies showed that M. avium subsp. paratuberculosis remained culturable in lake water microcosms for 632 days and persisted to 841 days. Of four reservoirs controlling the catchment area of the Taff, M. avium subsp. paratuberculosis was present in surface sediments from three and in sediment cores from two, consistent with deposition over at least 50 years. Previous epidemiological research in Cardiff demonstrated a highly significant increase of Crohn's disease in 11 districts. These bordered the river except for a gap on the windward side. A topographical relief map shows that this gap is directly opposite a valley open to the prevailing southwesterly winds. This would influence the distribution of aerosols carrying M. avium subsp. paratuberculosis from the river.

This paper raises a number of important questions in relation to possible sources of infection for humans. Given the time taken for the isolates of M. paratuberculosis to appear in culture, coupled with the inability to culture isolates from some PCR positive samples, this would indicate to me a suboptimal culture procedure was used. The use of Herrold's egg yolk media results in particularly poor recoveries of the “ovine” types of M. paratuberculosis. In contrast, these strains grow very well in Bactec 12B medium supplemented with egg yolk. To the best of my knowledge the “ovine” strain of M. paratuberculosis has never been isolated from a Crohn’s patient. Is this because of the use of suboptimal culture procedures or are these strains less pathogenic for humans? A fascinating paper which I urge members to read.
Selected summaries: Mycobacterium in Crohn’s: Something to ruminate about.

R.D. Cohen


This article is a critique of the recent article by Naser and colleagues, “Culture of Mycobacterium avium subsp. paratuberculosis from the blood of patients with Crohn’s disease. Lancet 2004;364:1039-1044.

Cohen’s principal message is summarised in the last sentence of the article;

“Although regurgitation may be acceptable in the bovine herds susceptible to Johne’s disease, the medical community is at a point where studies must go beyond merely repeating the observation that MAP is more commonly found in patients with Crohn’s disease and must establish whether there is truly an infectious role of this agent.”

I suspect that this sentiment is held by virtually all of those who have an interest in Johne’s disease and Crohn’s disease. What Cohen failed to do in his article is to suggest any way forward that would help answer the question of “association” versus “causation”. A better understanding of the pathogenesis / immunology of Crohn’s disease could well provide an answer to this question. Thus, Cohen’s challenge should be directed as much to the gastroenterology community as to those studying Johne’s disease. Editor.

Injury caused by self-inoculation with a vaccine of a Freund's complete adjuvant nature (Gudair) used for control of ovine paratuberculosis.

Windsor PA, Bush R, Links I, Eppleston J.


Farm Animal Health, Faculty of Veterinary Science, University of Sydney, PMB 3, Camden, New South Wales 2570.

OBJECTIVE: To document the occurrence and consequences of accidental self-inoculation of vaccinators (producers, farm employees, contractors) with the recently registered Gudair vaccine for the control of ovine paratuberculosis in Australia.

DESIGN AND PROCEDURE: A survey of the first 50 primary producers permitted to use the vaccine in sheep and a description of six cases of accidental self-inoculation for which medical attention was sought, and which occurred after the vaccine became widely available. RESULTS: The survey recorded that, of 37 respondents vaccinating 155,523 sheep, there were 21 incidents of exposure to the vaccine, an overall rate of one incident per 7,406 vaccinations. In five of these incidents there was only superficial skin contact with vaccine; in 16 there was needle penetration without vaccine injection. There were no reports of self-inoculation with vaccine. Six cases of
self-inoculation with Gudair vaccine that required medical intervention are described. Of these five were in males and one in a female; four involved injection of vaccine into the leg and single cases involved a foot or hand. Most cases required surgical removal of the injected vaccine to allow wound repair; three required extensive surgery and open drainage. Even with surgery recovery took as long as 9 months. Possible risk factors for self-inoculation and the resulting outcome are discussed. CONCLUSIONS: Gudair ovine paratuberculosis vaccine can cause prolonged granulomatous inflammation if inadvertently injected into human tissue. After self-inoculation, early surgical debridement of the damaged tissue and drainage to remove the vaccine material are advised to avoid progression to extensive necrosis.

Editor: This is an important paper which highlights one of the major disadvantages of “oil and whole mycobacteria” Johne’s vaccines. This type of vaccine can also cause significant lesions in sheep. The acceptability of a vaccine is sometimes a fine balance between the adverse side effects and the benefits. Recent studies in Australia have shown that the Gudair vaccine provides significant levels of protection against Johne’s disease in sheep. It is likely that vaccination will become an important tool for controlling clinical Johne’s disease in Australia.

Vaccination of sheep against M. paratuberculosis: immune parameters and protective efficacy.

Begg DJ, Griffin JF.

Vaccine, 2005, In press

Disease Research Laboratory, Department of Microbiology and Immunology, University of Otago, P.O. Box 56, Dunedin, New Zealand.

Johne's disease in ruminants is caused by the pathogenic bacterium Mycobacterium avium subspecies paratuberculosis (Map). Currently available Map commercial vaccines protect against clinical disease but not infection. In this study, the proprietary Johne's vaccine Neoparasec™ and an aqueous formulation of Map 316F (AquaVax) were tested in sheep. Detailed immunological examination of blood and gut-associated lymphoid tissues was carried out on animals after vaccination and challenge with virulent Map to identify markers of protective immunity. Neoparasec™ mark vaccination provided significant protection against disease while AquaVax did not. Immune animals had stronger cell-mediated responses and altered proportions of CD4(+), CD8(+), CD25(+) and B cells in blood, spleen and the gut lymphatics, than diseased animals.

Editor. This is one of the first papers where a comprehensive range of immune parameters have been measured in a controlled vaccination-challenge trial. Information from this and similar trials will be valuable for the development of a new generation of vaccines to control paratuberculosis.
Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom.

Stott AW, Jones GM, Humphry RW, Gunn GJ.

Veterinary Record 2005, 156, 825-31.

Epidemiology Research Unit, Animal Health Economics Team, sac, Craibstone Estate, Aberdeen ab21 9ya.

This paper estimates the financial incentive to control paratuberculosis on dairy farms by establishing the level of expenditure that would minimise the total cost of the disease (output losses plus control expenditure). Given the late onset of the clinical signs and the lack of treatments, control was focused on minimising the financial impact of paratuberculosis by adjusting the dairy cow replacement policy. The optimum replacement policies for disease-free herds and infected herds were compared by using dynamic programming. At the standard settings, the disease justified adjusting the culling policy; under constant bioeconomic assumptions, it reduced the expected annuity from milk production under the optimal replacement policy by about 10 per cent (pound27 per cow annually), a considerably lower figure than for other major endemic diseases that affect dairy cows in the UK. The effect was even less at lower milk prices, suggesting that there is at present little incentive for dairy farmers to put more resources into controlling the disease. However, the incentive could be increased if more information were available about how best to manage the disease under specific farm circumstances. Any effect that paratuberculosis may have on the future demand for milk and hence on milk prices would also be an important consideration.

Editor. I suspect that the conclusions from this study would also apply to other countries. The unresolved Crohn’s issue has a major influence on whether or not there will be a change in the financial incentives to control paratuberculosis.
For those who are not attending the 8ICP I recommend a visit to the website to view the abstracts [http://www.paratuberculosis.org/proc8](http://www.paratuberculosis.org/proc8).

A proceedings will be published which members can purchase at a reduced rate.
Genomics and molecular genetics of *Mycobacterium avium* subsp. *paratuberculosis*, a veterinary pathogen with zoonotic potential

A special session was devoted to paratuberculosis at this prestigious conference.

"**Objective:** *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne’s disease (paratuberculosis), an illness affecting all ruminants and possibly wildlife. It has been suggested that MAP may play a role in Crohn’s disease, a chronic inflammatory bowel disease in humans. Progress has been made in recent years in the development of molecular tools to study the mechanisms of pathogenesis. Recently the MAP genome has been sequenced opening the possibility for post-genomic studies to unravel host-pathogen interactions with the ultimate goal of developing better diagnostic and control strategies. This symposium will focus on initial studies flowing from the genomic sequence, new developments in molecular genetic studies, and recent developments in molecular strain typing of this important pathogen. Attendees will learn about the latest developments in MAP research and how this can be used to identify determinants of pathogenicity."

Conveners: R.G. Barletta and J.P. Bannantine.

Presentations:

J.P. Bannantine. The MAP genome and its initial application to MAP research.


J.M. Inamine. Proteomic and lipidomic analysis of MAP and insights into the mechanism of pathogenesis.

S. Sreevatsan. Molecular epidemiology of MAP.

Copies of these presentations will be available from the American Society for Microbiology.
For those interested in Johne’s disease control programmes this is a website that you should regularly visit. Much can be learned from Australia on the control of this disease.


Quarterly Newsletter - JD News

Summer 2005

Johne’s Disease – nomenclature

The following is an extract from “A Monograph on Johne’s Disease (Enteritis chronica pseudotuberculosa bovis) written by F.W. Twort and G.L.Y. Ingram in 1913. Twort was the first to grow the “Johne’s bacillus” and reported his findings in the following paper, “A method for isolating and growing the lepra bacillus of man and the bacillus of Johne’s disease in cattle (preliminary note), Vet J. 1910, 67:118-120”. With the advantage of hindsight, the claims for growing the “lepra bacillus” were obviously very premature, since nearly 100 years later a method for the in vitro culture of this organism has not yet been discovered. Editor.

Johne’s Disease
Chapter 1

Nomenclature, definition, history, and distribution


French. - Enterite specifique chronique des boeufs.

German. - Die spezifische chronische Enteritis des Rindes. Die chronische pseudotuberkulose Darmentzundung des Rindes.

Danish. - Kvaegts kroniske smitsomme Tarbetaendelse. Lollandske Syge.

Swiss. - Kaltbrandigkeit.

In Great Britain the disease to be described in this book is usually spoken of as “Johne’s disease”- from the name of the discoverer of the acid-fast bacillus present in the lesions of cattle – as being less cumbrous than the more descriptive term, “chronic pseudo-tuberculous enteritis,” which is commonly used in other countries. The latter name was suggested by Professor B. Bang in 1906, in which year he showed that the condition was a distinct disease in no way connected with tuberculosis; so that it has really more claim to be called “Bang’s disease,” and it causal micro-organism “Johne’s bacillus.”

An objection to the term “pseudo-tuberculous enteritis” exists in the fact that there is never any resemblance between the macroscopic lesions of Johne’s disease and those of tuberculosis, though under the microscope it is rarely possible to differentiate the causal micro-organisms. However, though for more than ten years after the discovery of acid-fast bacilli in the lesions the disease was regarded as a from of tuberculosis, and although the thickening of the bowel had been noticed by other observers previous to Johne and Frothingham, yet the name “Johne’s disease” has been adopted in England, and nothing would be gained by attempting to alter it. In America it is
known as “chronic bacterial dysentery,” to which it may be objected that the passage of blood with the faeces is not a very common feature of the disease.

The popular terms for this disease are somewhat varied, and differ in different localities – “skitters,” “scanters,” “piners,” and “wasters,” are all terms applied to animals showing emaciation and diarrhoea. Possibly the term “waster” is more commonly applied to tubercular animals, in which the diarrhoea is less marked; but no reliance can be placed on the use of these terms by farmers.

“Scrapy” seems to be a term somewhat loosely applied to the disease when it affects sheep. Stockman applied this term in his various articles on the subject, but states that it may be the irritable skin condition to which reference is made under this name, an which in sheep seems to be associated in some cases with bacterial enteritis.

“Lollandske Syge,” or Lolland disease, is the old name for the disease in Denmark, arising from the prevalence of the condition in cattle on that island.

“Kaltbrandigkeit,” according to Meyer, is the common term for Johne’s disease among Swiss farmers, in whose stock it occurs. This term merely describes the symptoms of “thirst without feverishness”.

It should be noted that “Twort and Ingram” do not refer to “paratuberculosis” in the section of their monograph relating to the nomenclature of the disease. However, “paratuberculosis” or more correctly, the “paratuberkelbazillus” is referred to in Chapter VI

Chapter VI
Description of Johne’s bacillus – cultivation of the bacillus – nature of the “essential substance” – description of cultures

“Description of Johne’s bacillus. – The specific bacillus of pseudo-tuberculosis enteritis, commonly known as “Johne’s bacillus,” belongs to the acid-fast group of bacteria, and is allied to the various tubercle bacilli. According to the classification of micro-organisms adopted by Lehmann and Neumann, it would be more correct to describe it as a mycobacterium, and the scientific name of the micro-organism would then be Mycobacterium enteritidis chronicae pseudotuberculoae bovis Johne, the name by which we have suggested it should be known. At the same time, in English literature it is usually referred to as “Johne’s bacillus” and in Continental countries as the “paratuberkelbazillus” or bacillus of pseudo-tuberculous enteritis………………

In the first edition of Bergey’s Manual of Determinative Bacteriology (1923) the organism was formally named as Mycobacterium paratuberculosis. Editor.