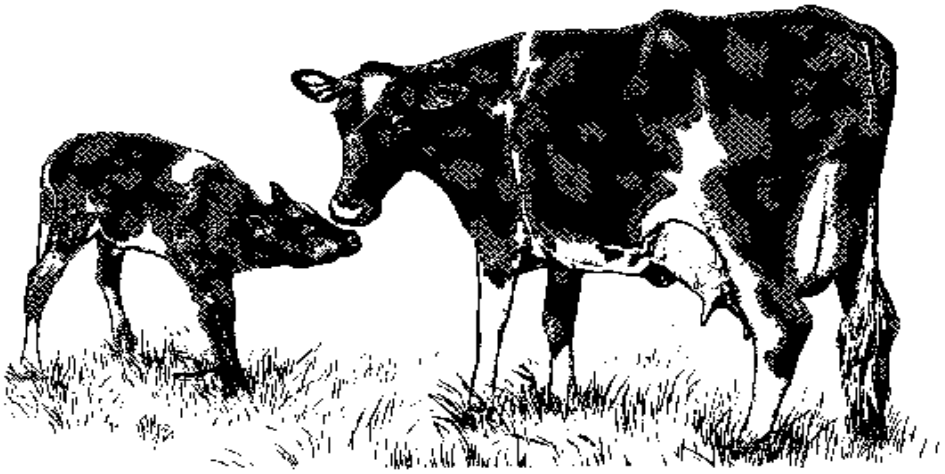
The Paratuberculosis Newsletter - Online

September 2004



An Official Publication of the INTERNATIONAL ASSOCIATION for PARATUBERCULOSIS and Other Intestinal Mycobacterioses

Editor's notes

The online editions of the Newsletter are slowly evolving. What are likely to become a more prominent feature of the Newsletter are weblinks to information that members of the Association may find interesting. The web contains a mind-boggling amount of information that is just sitting there waiting to be read and assimilated. The problem is that there is just far too much information and it is necessary to be very selective in what one reads. I see an increasing role for the Paratuberculosis Newsletter as highlighting for members information that they may find interesting and useful. In this regard I have provided a list of web sites for commercial firms selling reagents and services associated with paratuberculosis.

My selection of recent publication highlights my prejudices and interests in paratuberculosis. I am sure that many members, myself included, will have found the literature on the thermal inactivation of *M. avium* subsp. *paratuberculosis* confusing. What is apparent is that pasteurization kills this organism. The debate has more recently been focused on whether there is sufficient killing to have a high probability of removing all viable *M. avium* subsp. *paratuberculosis* from commercially pasteurized milk. The recent paper by O'Reilly and colleagues from Ireland found DNA evidence of *M. avium* subsp. *paratuberculosis* in commercially pasteurized milk samples but no viable organisms were identified. Their conclusion was that "since no viable *M. paratuberculosis* was isolated from commercially pasteurized cows' milk on retail sale in the Republic of Ireland, current pasteurization procedures are considered to be effective". Members are urged to read this paper as well as that of Stabel et al. who addressed the problem of on-farm pasteurization of milk for feeding to young calves.

In recent years Australia has been at the forefront of implementing programmes for the control of paratuberculosis. One of the motivating factors for instituting such programmes was the belief that in some parts of the country the disease was either absent or at extremely low levels. There was a hope that Western Australia and Queensland could be designated as being "paratuberculosis free". Recently, paratuberculosis has been detected in 18 sheep flocks in Western Australia. Epidemiological investigations indicate that the infection has been in some flocks for many years, consequently eliminating the possibility of achieving "paratuberculosis freedom" status in short to medium term. Many valuable lessons can be learnt from the Australian paratuberculosis control programmes. This latest setback is a reminder of the difficulties of identifying infected flocks or herds, especially where the prevalence of infection is low.

The Paratuberculosis Newsletter is happy to publish articles by members. These can be highly speculative and challenge the current dogma of paratuberculosis. Some of this dogma is not supported by robust evidence and merits re-examination. The article by Ian Lugton challenges some of the currently held beliefs as does the recently published paper by Leigh Corner and colleagues on the respiratory tract as a possible route of infection for paratuberculosis.

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Message from the President:

September 14, 2004

The paratuberculosis information explosion continues unabated. A quick search of literature databases using the search term "paratuberculosis" for only years 2003 and half of 2004 found 177 items using CAB abstracts and 147 using Medline. This verifies what we all feel, I am sure: our field of study is expanding at a tremendous rate and staying current with new literature is becoming increasingly difficult.

The International Association for Paratuberculosis provides a way to interact with colleagues with similar interests and stay abreast of this accelerating and exciting field of research. And, the Association is working to make the task easier. This month we contracted with a company, Xhaus.com, to upgrade and expand the features available on the Association's website http://paratuberculosis.org/

An important new feature to be up and running very soon is abstract management for our regular meetings. We hope that this service will provide a simple, consistent system for contributors to our regular colloquia to submit papers. Watch the website and future announcements about abstract submission deadlines for the 8ICP to be held in Copenhagen August 2005. If you visit the Association's site now you will find a link to the 8ICP website where more details about the meeting have been provided by our Danish hosts.

Other features to be added to the Association's website are expanded membership management, delivery of Newsletters and management of proceedings publication. These services will make the Association more effective and efficient. Remember also that you have a personal page on the Association's site where you can let the world know of your research and special interests at no cost. It is also a great way to connect with your colleagues and find those who are working on the same aspect of paratuberculosis.

Soon you will receive a call for nominations for officers and Governing Board members. Please take time to consider whether you would like to take a more active role in the business of the Association. The association is only as strong as its officers and Board members. The Board needs a constant infusion of new ideas and enthusiastic, hard working individuals.

On a more personal note, I am now on my fourth sabbatical. This sabbatical is made possible by a grant from the Fulbright Scholars Program. From August 2004 through July 2005 I am working at Universidad Austral de Chile in the city of Valdivia. This is in the heart of Chile's diary industry and I am enjoying the chance to work beside my Chilean colleagues to understand this disease and improve methods for diagnosis and control. I also hope to use this year to expand my language skills by learning to speak Spanish. This is long overdue, but the kind of learning opportunity that is perfect for sabbaticals.

In less than a year we will be gathered in Copenhagen to renew friendships and share the latest information from our research. I look forwarded to seeing you all again.

Sincerely,

Michael T. Collins
President, International Association for Paratuberculosis

8th International Colloquium on Paratuberculosis

August 14-18 2005, Copenhagen, Denmark

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http://www.8icp.dk /

The editor's selection

The following are a selection of recent publications that were of interest to the editor.

Extensive genomic polymorphism within Mycobacterium avium.

Semret M, Zhai G, Mostowy S, Cleto C, Alexander D, Cangelosi G, Cousins D, Collins DM, van Soolingen D, Behr MA.

Bacteriol. 2004 Sep;186(18):6332-4.

We have initiated comparative genomic analysis of Mycobacterium avium subspecies by DNA microarray, uncovering 14 large sequence polymorphisms (LSPs) comprising over 700 kb that distinguish M. avium subsp. avium from M. avium subsp. paratuberculosis. Genes predicted to encode metabolic pathways were overrepresented in the LSPs, and analysis revealed a polymorphism within the mycobactin biosynthesis operon that potentially explains the in vitro mycobactin dependence of M. avium subsp. paratuberculosis.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15342607

This paper provides a fascinating comparison of *M. avium* subsp. *paratuberculosis* and other members of the *M. avium* complex (Ed).

The respiratory tract as a hypothetical route of infection of cattle with *Mycobacterium avium* subspecies *paratuberculosis*.

Corner LA, Pfeiffer DU, Abbott KA.

Aust Vet J. 2004 Mar;82(3):170-3.

Department of Large Animal Clinical Studies, Faculty of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland. Leigh.Corner@UCD.IE

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15088986

A paper which challenges the dogma that transmission of paratuberculosis is principally by the faecal-oral route. While the editor is not convinced by the alternative hypothesis he encourages members to read this paper. Much of the current thoughts about paratuberculosis are based on inadequate information and the examination of alternatives to the dogma are most welcome.

Intrauterine and transmammary transmission of Mycobacterium avium subsp paratuberculosis in sheep.

Lambeth C, Reddacliff LA, Windsor P, Abbott KA, McGregor H, Whittington RJ.

Aust Vet J. 2004 Aug;82(8):504-8.

CONCLUSION: Although intrauterine or transmammary transmission of Mycobacterium avium subsp paratuberculosis may occur frequently in clinically affected sheep, these are less common in subclinically infected ewes. Therefore these modes of transmission are unlikely to compromise existing control programs for ovine Johne's disease on most farms, especially if programs include the immediate culling of clinically affected sheep.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15359967

Surveillance of Bulk Raw and Commercially Pasteurized Cows' Milk from Approved Irish Liquid-Milk Pasteurization Plants To Determine the Incidence of Mycobacterium paratuberculosis.

O'Reilly CE, O'Connor L, Anderson W, Harvey P, Grant IR, Donaghy J, Rowe M, O'Mahony P.

Appl Environ Microbiol. 2004 Sep;70(9):5138-44.

Food Safety Authority of Ireland, Abbey Court, Lower Abbey St., Dublin 1, Ireland. info@fsai.ie

Over the 13-month period from October 2000 to November 2001 (inclusive), the Food Safety Authority of Ireland (FSAI) carried out surveillance of Irish bulk raw (n = 389) and commercially pasteurized (n = 357) liquid-milk supplies to determine the incidence of Mycobacterium paratuberculosis. The pasteurization time-temperature conditions were recorded for all pasteurized samples. Overall, 56% of whole-milk pasteurized samples had been heat treated at or above a time-temperature combination of 75 degrees C for 25 s. All analyses were undertaken at the Department of Food Science (Food Microbiology) laboratory at Queen's University Belfast. Each milk sample was subjected to two tests for M. paratuberculosis: immunomagnetic separation-PCR (IMS-PCR; to detect the presence of M. paratuberculosis cells, live or dead) and chemical decontamination and culture (to confirm the presence of viable M. paratuberculosis). Overall, M. paratuberculosis DNA was detected by IMS-PCR in 50 (12.9%; 95% confidence interval, 9.9 to 16.5%) raw-milk samples and 35 (9.8%; 95% confidence interval, 7.1 to 13.3%) pasteurized-milk samples. Confirmed M. paratuberculosis was cultured from one raw-milk sample and no pasteurized-milk samples. It is concluded that M.

paratuberculosis DNA is occasionally present at low levels in both raw and commercially pasteurized cows' milk. However, since no viable M. paratuberculosis was isolated from commercially pasteurized cows' milk on retail sale in the Republic of Ireland, current pasteurization procedures are considered to be effective.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15345392

Destruction of Mycobacterium paratuberculosis, Salmonella spp., and Mycoplasma spp. in raw milk by a commercial on-farm high-temperature, short-time pasteurizer.

Stabel JR, Hurd S, Calvente L, Rosenbusch RF.

J Dairy Sci. 2004 Jul;87(7):2177-83.

The 2002 NAHM's Dairy Survey indicated that 87.2% of dairy farms in the United States feed waste milk to their neonatal calves. Although cost-effective, this practice can lead to increased calf morbidity and mortality due to ingestion of pathogenic agents. In an effort to reduce the risk of infection, dairy producers are implementing on-farm pasteurization of the waste milk as a control procedure before feeding the milk to calves. In the present study, the efficacy of a commercial high-temperature, short-time (HTST) on-farm pasteurizer unit to destroy Mycobacterium paratuberculosis, Salmonella enterica spp., and Mycoplasma spp. in raw milk was evaluated. Replicate experiments were run for 3 isolates of M. paratuberculosis, 3 serovars of Salmonella (derby, dublin, typhimurium); and 4 species of Mycoplasma (bovis, californicum, canadense, serogroup 7) at 2 different levels of experimental inoculation. In addition, HTST pasteurization experiments were performed on colostrum experimentally inoculated with M. paratuberculosis. After culture of the pasteurized milk samples, no viable M. paratuberculosis, Salmonella, or Mycoplasma were recovered, regardless of species, strain, or isolate. Pasteurization of colostrum was also effective in the destruction of M. paratuberculosis but resulted in an average 25% reduction in colostral immunoglobulin. These results suggest that HTST pasteurization is effective in generating a safer product to feed to young calves.

Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease.

Naser SA, Ghobrial G, Romero C, Valentine JF.

Lancet. 2004 Sep 18;364(9439):1039-44.

”We detected viable MAP in peripheral blood in a higher proportion of individuals with Crohn's disease than in controls. These data contribute to the evidence that MAP might be a cause of Crohn's disease.”

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15380962

This paper is likely to generate a considerable amount of interest in the debate on whether or not *M. paratuberculosis* has a role in the cause of Crohn’s disease (Ed).

Paratuberculosis – Commercial websites

The following are a list of website addresses that refer to sites of companies that are selling products and or services relating to paratuberculosis. The listing of the services is not an endorsement of them by the International Association for Paratuberculosis. The Association does not have an official opinion as to the value of the services or products described in the websites. Almost certainly, this is not a complete list and some sites may not be current. The editor encourages members to forward to him any relevant websites that are not included in the list below or any corrections of fact. These will be included in subsequent issues of the Newsletter.

Adiagen

http://www.adiagene.com/produits/mp.htm

Products

Adiavet^{R} Paratb and Adiavet^{R} Paratb real time tests. PCR and real time PCR kits for the examination of faeces or the identification of cultures.

Allied Monitor

http://www.alliedmonitor.com/index.html

Products

Ferric mycobactin J
Paratuberculosis protoplasmic antigen
Positive and negative control sera (bovine, caprine and ovine) for use as controls in ELISA and AGID against Allied PPA antigen.
Absorben (*M. phlei*), a whole-cell, heat killed, lyophilised *Mycobacterium phlei* for absorbing test sera prior to testing with ELISA.

Services

Serum ELISA
Milk ELISA
Agar gel immunodiffusion
Faecal culture

Antelbio

http://www.antelbio.com/Press_Room/two_tools_for_detection_of_johnes.htm

Services

Faecal culture
Serum ELISA
AntelBio Rapid fecal test (PCR)
AntelBio Johne's milk ELISA

Becton Dickinson (BD)

http://www.bd.com/Industrial/products/qaqc/veterinary/herrolds.asp

Products

BBL™ Herrold's egg yolk medium with mycobactin J
BBL™ Herrold's egg yolk medium without mycobactin J

Biocor

http://www.biocorah.com/diag/para.shtml

Products

Paracheck™ an absorbed ELISA for use in cattle, sheep and goats
Bovigam™ an interferon gamma test originally developed for the diagnosis of bovine tuberculosis. This test has been widely used in investigations on paratuberculosis in cattle, sheep and goats.

Biotype AG

http://www.biotype.de

Products

Bactotype® Detection kit. A *Mycobacterium paratuberculosis* stool test using a PCR.

CSL

http://www.csl.com.au/pageManager.asp?pageID=118&countryid=0&departmentID=6&categoryid=7&DiseaseID=171

Products

Gudair[R] paratuberculosis vaccine, registered in Australia for the control of paratuberculosis in sheep.
Bovigam[TM] an interferon gamma test originally developed for the diagnosis of bovine tuberculosis. This test has been widely used in investigations on paratuberculosis in cattle, sheep and goats.

Defra

http://www.defra.gov.uk/corporate/vla/comserv/comserv-product.htm (VLA)

Products
Johne's disease vaccine

Gudair

http://www.czveterinaria.com/gudairin.html

Products
Paratuberculosis vaccine for goats and sheep

Guildhay

http://www.guildhay.co.uk/sitemap.htm

Products
Paratuberculosis ELISA serum screening
Paratuberculosis ELISA serum verification
Paratuberculosis Mycobactin J
Paratuberculosis PCR test kit
Paratuberculosis Realtime PCR test kit

Idexx

http://www.idexx.com/production/ruminant/ruminant4.cfm

Products

Herdchek[R] *Mycobacterium paratuberculosis* test kits
Mycobacterium paratuberculosis DNA probe. PCR developed for the examination of faecal samples
Mycobacterium paratuberculosis antibody ELISA (Absorbed ELISA)

Immuncell

http://www.immucell.com/pf/prod_bio.php

Products

rjt, a *Mycobacterium Paratuberculosis* Antibody Test Kit, is an immunodiffusion test specifically designed to aid in the differential diagnosis of cattle with clinical Johne's Disease.

Ribotechnologies

http://www.ribotechnologies.com/

Products
Parascan. Realtime PCR test for the detection of *Mycobacterium paratuberculosis* in faeces
http://www.microscreen.com/News/para.pdf

Pourquier

http://www.institut-pourquier.fr/va/diagnostic/gammesAnimal.html

Products

Absorbed ELISA kits
Mycobactin J

Svanova

http://www.svanova.com/filearchive/Manual%20Para-TB-Ab.pdf

Products

Paratb –Ab – ELISA kit for the detection of specific antibodies in bovine serum and milk.

Ovine Johne's disease in Western Australia

In November 2003, signs consistent with ovine Johne's disease (OJD) were seen in a line of sheep slaughtered at a southern abattoir and examined as part of Western Australia's OJD abattoir surveillance program. OJD was confirmed on histology and DNA typing.

The Department quarantined the property, which was a large sheep and cattle property in the Williams shire. Further testing confirmed further cases of OJD and provided the first evidence of OJD spread in Western Australia.

The Department established the cases an emergency incident and the incident team was tasked with checking neighbours and traces for signs of OJD. There are currently about 100 neighbour and trace properties associated with the incident.

Up to February 2004, OJD had also been diagnosed in one neighbouring property and five other properties that had received sheep from one or other of the two neighbouring properties. The further cases were diagnosed by targeted necropsies of old and thin sheep or by abattoir collection. Pooled faecal culture samples were also collected with first results due in mid April 2004.

The available evidence indicates that OJD has been present in WA for seven or more years and that none of the known infected flocks is the source of the disease in Western Australia.

The above is an extract from the March 2004 issue of the Animal Health Newsletter, Department of Agriculture, Government of Western Australia. The complete text can be found at;

http://agspsrv38.agric.wa.gov.au/pls/portal30/docs/FOLDER/IKMP/PW/AH/AHN200403.PDF

OJD update – new path set forward for OJD management in WA

The following is an extract from the June 2004 Animal Health Newsletter, of the Department of Agriculture, Government of Western Australia.

OJD has been diagnosed on seven more properties in WA, bringing the total number of properties with positive tests to eighteen. The results confirmed the assessment by Department veterinary staff that the disease is established in WA and that more infected properties are likely to be detected. ……

The complete text can be found at

http://agspsrv38.agric.wa.gov.au/pls/portal30/docs/FOLDER/IKMP/PW/AH/AHN200403.PDF

OJD Update – risk based trading in WA

Fiona Sunderman, Senior Veterinary Officer, Department of Agriculture, WA.

The Department of Agriculture and the sheep industry in Western Australia are conducting a communication program to assist farmers adjust to the new environment of Ovine Johne's disease (OJD). The risk based trading approach, endorsed by the OJD Advisory Committee, appropriately puts the responsibility for biosecurity measures and risk and disease management**,** with individual sheep owners and their agents, advisors and stock carriers.

Properties known to be infected with OJD were released from quarantine restrictions in early July 2004 and infected properties in the future will not have restrictions on movement within the State. The Department will notify properties detected with OJD through abattoir surveillance. However, any tracing and notification of neighbours or trace properties will be the responsibility of the owner of the infected property.

Deciding on the most appropriate prevention or control measures for an individual property eg vaccination, will be a business decision based on an assessment of the property's risk or level of infection, the flock structure, management and markets, and the owner's aversion to or acceptance of risk.

The most likely cause of infection in a flock is through the introduction of sheep. Introduced sheep, whether purchased, straying or agisted, can shed the bacteria in their faeces for years before the disease becomes apparent.

To assess the OJD risk of a sheep flock, farmers need to know its current status, its testing history, its trading history and its vaccination status. The OJD Animal Health Statement (AHS) provides most of this information. This document and a guide on it's use can be downloaded from the websites of Elders, Landmark, WAFarmers, PGA, Stud Merino Breeders' Association and the Department.

Abattoir surveillance on 1,000 lines of sheep is conducted in Western Australia each year and underpins the State's Area Prevalence status.

The OJD Advisory Committee recommends that owners consult their veterinarian on the most appropriate biosecurity risk management procedures for their enterprise. They can also consult their farm management advisor on the level of economic investment to manage their level of risk or disease.

An OJD Information Pack, containing a series of five fact sheets, includes the AHS and a guide on how to fill out the statement, and is available from the Department's Regional Offices or from the Department of Agriculture's website at www.agric.wa.gov.au
The Information Pack is also available from private veterinarians or local stock agents.

AnimalHealth AUSTRALIA

Johne's Disease Information Centre

DCP National Coordinators Quarterly Report

Quarterly Newsletter - JD News

- **Volume 5 Number 2** - Autumn 2004 [PDF] 365KB ***LATEST***

http://www.aahc.com.au/jd/NJDCP%20QRep%20June%2004.pdf

Micronutrients, cell-mediated immunity and the clinical expression of disease caused by *M a paratuberculosis* and other pathogenic mycobacteria: a review

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Preamble

This article is one of a series of three which I have written to explore the epidemiology of paratuberculosis and in which I put forward a new epidemiological paradigm (or perhaps reinvigorate an old one) regarding this disease. I believe that the time is right to review the fundamental way we think about this disease as by pursuing the current paradigm little progress in control has been made, and it would seem that in many areas that expression of the disease is worsening.

The first of these papers has been published in the June edition of the Australian Veterinary Journal (AVJ).[1] This paper suggested that light infertile soils with improved clover pastures were an important risk factor for promoting disease progression in sheep. The recovery rate or the failure of the disease to progress also appeared to be important in limiting expression. Factors that were not found to be important were the time the infection had been present, the stocking rate and by implication the infectious load in the environment. Satisfaction of gross nutritional requirements also seemed to have little effect in preventing disease progression.

The second paper will be published in the August edition of the AVJ.[2] This paper explores and reviews the literature concerning possible links between the clinical expression of paratuberculosis, soils and micronutrients. This review provides strong evidence implicating the involvement of acidic soils that predispose to nutritional excesses of iron and molybdenum and deficiencies in copper and selenium and possibly other minerals and trace elements as having a key functional role in the disease process. However, the review stops short of examining plausible explanations of how these nutritional factors might operate within the host. Because I feel that it is necessary to provide these plausible explanations to further develop and explain the hypothesis I have produced a third manuscript.

This third paper provides supporting evidence in addition to that provided by experimental, epidemiological and field studies that implicate the involvement of micronutrients in the clinical expression of mycobacterial diseases. However, as this article shows bias and is somewhat speculative it has been unacceptable for publication in the AVJ. So that my intellectual effort is not wasted and the ideas are put forward within the scientific community, I have chosen to print this manuscript in the Paratuberculosis Newsletter.

Some may find the papers confronting, others may find that they provide explanations for hitherto inexplicable observations. I would like to believe that these manuscripts will generate more light than heat and will lead to the unearthing of more truths about this disease which has historically proven somewhat difficult to study. The views expressed are mine and not those of my employer.

Introduction

For optimal physiological and immunological function and suppression of infection, the composition of the mineral fraction of the diet must be sufficient and balanced. These optimal dietary conditions, which researchers are now coming to understand do not coincide with those for greatest growth or reproduction, should assist hosts to eradicate the infecting bacilli or at least promote the development of a dormant infectious state. Lack of detailed knowledge of the actual effect of these nutritional factors begs for more research directed at better understanding of the role of these elements and their actual involvement in the clinical expression of paratuberculosis and other mycobacterioses.

The pathogenic mycobacteria are obligate intracellular parasites that typically reside within macrophages. Early in the infection process invading mycobacteria take advantage of these phagocytes to gain entry to the intracellular milieu where, if they survive, they reside within phagosomes subverted into a state of arrested development.[3] In a resistant host, the engulfing phagosome goes through a process of maturation that results in the development of a

phagolysosome. As a result of the maturation process the phagosomal lumen becomes a highly acidic oxidising environment, filled with hydrolytic enzymes that under optimal conditions can effectively kill and digest most of its bacterial contents.[4,5] Within this potentially protected environment of the susceptible host's macrophage pathogenic mycobacteria may replicate and evade many of the body's cell-mediated immune (CMI) defences. Polymorphonuclear neutrophils have also been implicated in the early inflammatory response against mycobacteria in addition to monocytes and macrophages. These phagocytes undergo a cytokine-mediated activation at the site of mycobacterial infections and it is believed that this activation might set the stage for a subsequent antimycobacterial immune response.[6]

The aim of this paper is to examine what is known of the biological and immunological processes within the host and the requirements of mycobacteria that provide substantive support for the notion that micronutrient deficiencies or imbalances play a key role in allowing the clinical expression of paratuberculosis and other mycobacterioses. Much of the detail focuses on the effects of minerals and trace elements on CMI and the biochemical activity within host macrophages.

Biological function of minerals and trace elements in the host

Dietary deficiencies of minerals or factors that inhibit the utilisation of minerals will limit the ability of the immune system to deal with infectious organisms. Nutritional depletion or an imbalance of agonists and antagonists may adversely affect virtually all components of the immune system since minerals and trace elements are often essential components of enzymes that have crucial roles in many biochemical reactions. Nutritional deficiencies can have widespread effects on a great range of physiological processes. For instance, the antioxidative defence mechanisms employed by the body are dependent upon a range of vitamins, amino acids and trace elements including zinc and selenium.[7] Cells with a short half-life, like many of the leukocytes, are particularly sensitive to trace element deficiency or fluctuations in availability. Additional nutritional requirements accompany states of increased metabolic activity, as occurs during pregnancy, lactation, increased cellular proliferation and modulation of the immune system. During such times increased micronutrient demands can induce signs of mineral deficiency in animals with only marginally deficient diets.

Mineral requirements for ruminant production have been estimated, but little work has been published on any additional requirement for the maintenance of full immune function. Unfortunately there are few studies reporting the effects of mineral deficiency, interactions or supplementation on ruminant immunity or immune mediated protection against pathogens. Listed below are some essential minerals with summaries of their principal functions and any known role in maintaining the integrity of the CMI.

Phosphorous

Eighty percent of the body's phosphorous is in the skeleton. However, the remainder is critical for cellular energy metabolism, being a component of ATP, nucleic acids and phospholipids. Phosphorous controls many key regulatory enzymes. As a consequence of the key role of this element, low blood phosphorous levels have been shown to impair chemotactic, phagocytic, and bactericidal activity of granulocytes in dogs.[8] In pigs optimal levels of phosphorous have been shown to enhance CMI responses, including lymphocyte proliferation and the response to intradermally injected phytohaemagglutinin (PHA).[9,10]

Calcium

This element is known to be a universal secondary messenger, indispensable in the majority of cellular signal transduction pathways, including nitric oxide production and the induction of cell death. It is also involved with blood clotting, muscular contraction and transmission of nerve impulses. Increased cytosolic calcium concentration is an early event following phagocytosis because calcium is involved in signalling cascades that activate kinases, induce cytoskeletal changes and the development of phagolysosomes.[11]

Iron

Iron is involved in oxygen transport and storage (haemoglobin and myoglobin), electron transport and is a cofactor in numerous enzymes such as catalase, myeloperoxidase, tryptophan 5-monoxygenase, phenylalanine 4-monoxygenase and aconitase. Iron deficiency decreases phagocyte function, lymphocyte proliferation and natural killer cell activity.[7]

Molybdenum

Molybdenum is an essential trace element in that it is a component of several enzymes including xanthine oxidase, aldehyde oxidase and sulphite oxidase. Xanthine oxidase is involved with purine metabolism and produces O_2^- and H_2O_2 as a by-product of this process.

Optimal concentrations of molybdenum, higher than those required for productivity, have been shown to enhance the resistance of sheep to secondary challenge from nematode larvae.[12] Such an effect was thought to act through an impact on acquired CMI through enhancing the inflammatory response to nematodes by increasing O_2^- concentration in the mucosa. This effect may work directly or, indirectly by reducing the effectiveness of the local copper-dependent anti-inflammatory enzyme, superoxide dismutase (SOD).[12]

Cobalt

This element is essential for ruminants because it is incorporated into vitamin B12 by rumen microbes. Vitamin B12 is required to metabolize propionate. In addition to the commonly accepted effects of cobalt deficiency that include the loss of appetite, rough hair coat, stumbling gait and anaemia, the immune system may also be impaired. A number of CMI functions are diminished by cobalt deficiencies[13] and in particular, lymphoproliferative responses to Johnin have been shown to be diminished in cobalt-deficient sheep vaccinated against *M a paratuberculosis.*[14]

Copper

Copper is a component of numerous enzymes including lysyl oxidase, tyrosinase, caeruloplasmin, cytochrome oxidase and SOD. Lysyl oxidase is involved in the cross-linking of connective tissue proteins including elastin which is especially important in maintaining the integrity of the aorta. Tyrosinase is involved in the production of the pigment melanin, and dopamine. Caeruloplasmin is essential for the normal flux of iron through the reticuloendothelial system, the liver and blood. Cytochrome oxidase is involved in the production of myelin.[15]

The site of uptake for copper is the lower ileum in sheep and the absorption of this element appears to vary between breeds.[16] Copper deficiency normally occurs through insufficient dietary intake or through competitive interactions with other elements.

There is evidence that the immunocompetence of copper-deficient animals is less than that of copper-replete animals. Several researchers have reported that copper deficiency alters non-specific CMI functions by tending to decrease phagocytic activity and neutrophil killing ability associated with low SOD activity.[17] This has been demonstrated *in vitro* by a reduction in the ability of granulocytes in sheep and cattle to kill *Candida albicans.*[18,19] Copper deficiency has also been found to result in increased mortality in rats infected with the intracellular parasite *Salmonella typhimurium*, when compared with copper-replete animals.[20] However, despite copper having some role in supporting CMI function, specific delayed-type hypersensitivity (DTH) reactions and lymphocyte blastogenesis have not been shown to vary consistently in copper-deficient cattle.[21,22]

Zinc

Zinc is a component of over 70 enzymes. Among these are alcohol dehydrogenase, DNA polymerase, RNA polymerase, carbonic anhydrase, carboxypeptidase A and B and pyruvate dehydrogenase. Zinc is involved in gene expression, membrane stability and is essential in Vitamin A metabolism. A deficiency of zinc results in decreased phagocytosis, reductions in circulating T cells and generally compromises CMI responses.[7,23] The immunomodulatory role of zinc is evident clinically through reduced tuberculin skin test reactivity in deficient subjects.[24] Clinical cases of human tuberculosis have also been found to possess lower plasma zinc levels than controls and to have improved treatment outcomes when supplemented with zinc.[23,25]

Trace elements like zinc and selenium have optimal dosages beyond which immune-mediated processes, such as phagocytic activity decline.[7] For example, even though adequate levels of dietary zinc are required for proper CMI function, elevated zinc levels in human hair have been associated with clinical tuberculosis. This suggests that either a deficiency or surfeit of zinc will adversely affect the outcome of clinical tuberculosis and possibly other mycobacterioses.[26]

Manganese

Transition metal ions, such as manganese, are essential to life and participate in many cellular functions including the regulation of transcription through DNA binding proteins and metal response elements, the functions of hundreds of enzymes including metalloproteases, SOD, inducible nitric oxide synthase, and even cellular functions such as endosomal fusion.[27] Manganese is an enzyme component in pyruvate carboxylase and arginase. Copper and zinc, as well as manganese, are functional components of SOD and dietary deficiencies of these elements

decrease SOD activity and increase levels of O_2^-. Inducible nitric oxide synthase is involved in the production of nitric oxide which is regarded as a key reactive nitrogen intermediate in antibacterial defences and may act synergistically with H_2O_2 in killing or inhibiting the multiplication of mycobacteria.[28]

Selenium

Selenium has a role in various important biological processes, such as iron metabolism, thyroid hormone metabolism, cell growth, eicosanoid biosynthesis, sperm development and protection from neoplastic processes, cardiovascular disease and oxidative stress.[29] There are at least 30 known selenium-containing proteins including deiodinases, selenoproteins P and W and glutathione peroxidase (GPx).[29,30]

One of the principal functions of selenium is through the production of four forms of GPx and their role in preventing cell and membrane damage induced by reactive oxygen intermediates (ROI). The various forms of GPx provide an extensive system of antioxidant activity in intra and extra-cellular sites as well as in the cell membrane itself.[30] The activity of cellular SOD such as copper-zinc containing and manganese containing dismutase is to reduce the concentration of O_2^-in the cytosol and mitochondria. These SODs neutralise or reduce the effects of oxidants by catalysing O_2^- to H_2O_2. However, in so doing they produce potentially toxic H_2O_2. GPx is the principal destroyer of both H_2O_2 and organic hydroperoxides. However, catalases containing iron or other transition metal ions also assist in this process, as do peroxidases. Selenoproteins P and W also have antioxidant and transition metal-binding properties that appear to play critical protective roles by limiting the production of ROI.[29]

In a review of selenium deficiency in grazing animals, Wichtel noted that there were few field observations associating selenium deficiency to increased susceptibility to infectious disease.[31] However, in selenium-deficient cattle phagocytes, GPx activity declines and this is associated with decreased microbicidal activity and reduced lymphocyte proliferation. Studies in other animals have shown that these GPx-deficient macrophages release more H_2O_2 and that immune-mediated cytotoxicity is reduced. In support of these observations, human studies have found significant increases in cytotoxic lymphocyte and natural killer cell activity following selenium supplementation.[32]

Micronutrients and phagocytes

Iron plays a key role in the balance between mycobacterial survival and host defence strategies. A delicate balance in body iron must be maintained for resistance to infections. Insufficient levels may impair antimicrobial defences expressed by macrophages and monocytes, including phagocytosis, cytokine production, myeloperoxidase activity and the respiratory burst resulting in the generation of ROI and halides.[33,34] Excess iron may have detrimental effects on host defences through increased availability of iron for mycobacterial antioxidative defences or by direct impairment of phagocytic activity, the formation of multinucleated giant cells and the production of nitric oxide.[35] It has also been reported that the damaging effects of H_2O_2 in hepatocytes, cardiac monocytes and erythrocytes is iron dependent.[29]

Bacteria have evolved a complex web of biosynthetic and metabolic pathways by which to obtain, store and regulate the use of iron. This is because iron serves as an essential bacterial nutrient. For example, in *M tuberculosis* iron is a cofactor for at least 40 different enzymes encoded in the genome.[36] An excellent review of the metabolism and role of iron in mycobacteria and in particular *M. tuberculosis* has recently been produced by Ratledge.[37]

To survive within monocytes and macrophages, mycobacteria have evolved mechanisms to either adapt to, or modify the hostile intracellular environment within the engulfing phagosome. Mycobacteria also persist within cells by resisting or neutralising the damaging effects caused by ROI, halides and reactive nitrogen intermediates generated by the host.[38,28,39] As part of this process it has been hypothesised that low iron response genes in mycobacteria may be generated in response to nitric oxide attack, and that this response could aid in the repair or replacement of damaged iron-containing proteins.[40]

Mycobacteria possess iron-containing catalase and types of SOD that in contrast to mammalian copper, zinc and manganese-containing SOD, contain only iron and manganese.[38] Although these mycobacterial catalase and SOD are likely to provide protection from host-generated ROI, they have not yet been identified in *M a paratuberculosis*.[33] However *M a paratuberculosis* does produce and excrete a ferric reductase that functions to acquire iron stored as ferritin within the cytoplasm of phagocytes.[33,41] It has been hypothesised that this secretion of ferric reductase during intracellular growth robs the host cell of iron, thereby protecting the bacteria from attack by toxic hydroxyl radicals, while also serving to scavenge iron to meet bacterial requirements. There is also some

evidence to suggest that mycobacteria use truncated haemoglobin-like proteins to promote resistance to attack by ROI.[39]

In a variety of bacterial pathogens, including mycobacteria, iron availability and sequestration within the host has been associated with virulence.[37,42] X-ray microscopy has been used to study iron levels within phagosomes infected with a virulent strain of *M avium*.[43] A significant increase in the concentration of iron was observed 24 hours after infection. However, with non-virulent *M smegmatis,* a decrease in iron concentration under the same circumstances was noted. Macrophages pre-stimulated with the protective cytokines interferon gamma or tumour necrosis factor alpha prior to infection with *M avium,* showed a significantly reduced iron concentration after infection, compared with untreated macrophages. Other studies with *M avium* have found that an adequate supply of iron is needed to prevent phagosome maturation within macrophages.[44] In humans it has been found that elevated dietary iron was likely to increase the risk of active pulmonary tuberculosis.[45] In this study it was proposed that the mechanism of action of the elevated iron intake was through the impairment of macrophage function but may also have been through the increased availability of transferrin to the mycobacteria. These studies provide firm evidence for the intra-phagosomal role of iron in allowing expression of mycobacterial virulence factors.

In addition to the previously listed effects of various elemental deficiencies or imbalances on components of CMI, there is another plausible mechanism of action whereby certain elements required by the bacilli may play a role in the pathogenesis of paratuberculosis. Genetic variants of Slc11a1 (formerly known as Natural Resistance-associated Macrophage Protein 1 or Nramp 1) have been shown in a number of species to be associated with resistance to infection by intracellular parasites such as mycobacteria and salmonellae.[46] Slc11a1 is expressed primarily in macrophages and polymorphonuclear leukocytes and is upregulated in these cells by exposure to activating or inflammatory stimuli. The studies suggest that Slc11a1 contributes to the defence against intracellular infection by extrusion of divalent cations, particularly iron and manganese, from the phagosome.[46,34]

These divalent cations are essential for microbial function and their removal from the phagosome impairs the virulence determinants of the invading intracellular bacilli that would otherwise prevent phagosome maturation and provide a site for initial replication.[4] Slc11a1 recruitment to the phagosomes of mycobacterium-infected macrophages is associated with increased bacterial cell damage and absence of bacillary replication.[34] However, the method by which Slc11a1 metal transport at the phagosomal membrane modulates either pathogen virulence or affects antimicrobial defences of phagocytes is not fully understood.[34]

Such processes involving Slc11a1 might, at least in part, explain the apparent association between diet, especially the intake of divalent cations like iron, copper, zinc, and manganese, and the clinical expression of paratuberculosis. The mechanism by which mycobacteria arrest phagosome maturation may depend upon manganese or iron dependent enzymatic activity that may be antagonised by Slc11a1 extractive transport at the phagosomal membrane.[34] Alternatively, the effect of Slc11a1 could be more general and secondary to deprivation of metal ions necessary for the metabolic activity of the bacilli. This bacterial metabolic activity may include the production of detoxifying metalloenzymes such as SOD.[47] Slc11a1-mediated deprivation of cations from the phagosome would impair this protective enzymatic activity and enhance the bactericidal effect of the phagocyte-generated ROI.

It has also been proposed that Slc11a1 may deliver divalent cations, such as iron, to the acidic phagosome where the Fenton reaction can use ferrous iron to generate toxic hydroxyl radicals that can be used to attack mycobacteria.[27] Because members of the Solute carrier family 11 group of proteins are expressed by both phagocytes and pathogens and are able to transport metals, they appear to play a key role at the intracellular host-parasite interface.

The condition of the mitochondria within *Mycobacterium*-infected macrophages has been proposed to be crucial to the outcome of infection, and controls whether the cell becomes necrotic or apoptotic.[48] Macrophage necrosis has been associated with increased bacterial growth, whereas apoptosis has been associated with effective antimycobacterial defences.[49] It has been proposed that the pathway to either apoptosis or necrosis depends upon the permeability of the mitochondrial membrane and that this permeability is controlled by the intracellular calcium concentration. Increases in calcium concentration within macrophages have been shown to protect mitochondrial integrity, assist phagosome-lysosome fusion, block necrosis and to assist effective antimycobacterial activity. [48,50] Elevated cytosolic calcium levels in macrophages have also been associated with an increased maturation of mycobacterial phagosomes and poor survival of the bacteria held within.[50] These findings suggest that deficiencies of calcium or an imbalanced calcium metabolism, such as those that frequently occur in the periparturient period in dairy cattle, might be one of the factors that precipitates the progression of clinical disease.

In addition to the role of calcium in controlling the fate of infected cells, selenium supplementation has also been found to induce apoptosis of neoplastic cells through mitochondrial membrane change mediated by production of ROI within cells.[51] This suggests that repletion with selenium may be necessary to properly direct apoptotic cell processes. Such activity might be beneficial in limiting mycobacterial infections as well as neoplastic processes.

Pathophysiology

Periparturience

There are increased requirements for trace elements and alterations in micronutrient balance at the end of gestation and the beginning of lactation because the foetus's requirements increase dramatically and many nutrients are also excreted into the colostrum and milk. This period is especially problematic for dairy cattle because of the high volumes of milk produced. There have been a number of studies reporting marked declines in serum concentrations of vitamins A and E and zinc, in addition to the well known imbalance in calcium metabolism at calving.[52] Trace element and antioxidant deficiencies are possibly a significant contributory factor in the well-recognised development of paratuberculosis in dairy cattle shortly after commencing lactation. The addition of selenium and vitamins A and E to the diet of dairy cattle has been shown to reduce the effect of CMI suppression involving the dysfunction of lymphocytes and phagocytes observed through the periparturient period.[53,54] Given that of all the domestic ruminants, dairy cattle are particularly recognised for developing clinical signs of paratuberculosis following parturition, it is likely that nutrient/metabolic imbalances occurring at this time may contribute significantly to the progression of disease.

Mycobacterial dormancy

Paratuberculosis is probably similar to other mycobacterial diseases in that once infection is present the bacilli can lie dormant in tissues either intact or as cell wall-deficient spheroplasts when the environment otherwise supportive of replication of the organism and its' survival becomes hostile.[55,56,57,58,59,60] This will occur to varying degrees depending upon the nutritional status of the animal and the capability of the CMI. It is likely that the bacilli can exist in a dormant state, often in the absence of any readily detectable immune response, and without being detectable or able to be grown in vitro.[59] Reactivation of growth and development of disease could follow immunosuppression.[61,62]

This state of dormancy and latent infection results in the situation where there is no satisfactorily sensitive method to show that any individual live animal (or human being) is free of infection. Even when a number of sophisticated tests are used, a large number of infected ruminants will not be identified as such.[63] Although this test insensitivity related to dormancy has featured in the debate about the role of *M a paratuberculosis* in the aetiology of Crohn's disease in humans,[64] it has not received much attention from those wishing to control or eradicate mycobacterial diseases from flocks or herds.

The host-parasite relationship in subclinical cases of paratuberculosis involving the carriage and periodic excretion of intracellular bacteria obviously suits survival of the parasite. However, it could possibly benefit the majority of hosts by continually re-stimulating CMI responses that prevent establishment of the bacilli and development of disease at other sites within the body. This comfortable relationship between potentially pathogenic mycobacteria and their hosts, the majority of which remain disease-free, is probably one of the major determinants of the success of these pathogens. This relationship ensures that there is always a pool of latently infected individuals in the population in which disease, bacillary excretion and transmission can occur following events which compromise the host's CMI.[65]

The demonstrated ability of carrier animals to exist in environments with soil types that do not predispose to the development of clinical paratuberculosis[2] and the current poor sensitivity of diagnostic tests raises concern about the possibility of infection being more widespread than previously believed. Such infection will be rarely recognised.

Site effects

Although it is tempting to speculate that it is the concentration of available nutrients or other competitive antibacterial factors in the gut that predispose to the development of paratuberculosis lesions in the bowel, there is no evidence to support this.[66] However, there is sufficient evidence to support the notion that changes in systemic micronutrient availability are involved in the pathogenesis of paratuberculosis without the further need to propose that the proximity of bowel lesions to ingested nutrients plays a significant role.

The organism circulates widely in the body and there are numerous anatomical sites and secretory routes where *M a paratuberculosis* can be isolated. It is likely that it is the inherent but unknown peculiarities of the ileal compartment of the immune system that allows the disease process to progress at this location. This site has evolved to be a convenient site for replication of these bacilli because it provides a ready pathway for both uptake and excretion of the organism, similar to the relationship between the lungs and tubercle bacilli in most hosts. However, the effects of micronutrient deficiencies or imbalances may be compounded by the existence of extensive lesions in the ileal mucosa. Such lesions may interfere with nutrient uptake, as proposed by Lepper et al[67] in the case of copper, as well as causing in the loss of proteins and other nutrients. Although this protein-losing enteropathy may make the clinical situation of any individual more precarious, it should be considered that it is the pre-existing nutritional deficiencies or imbalances that have already brought the immune system and the lesions in the animal to that point.

Epidemiological considerations

M a paratuberculosis is a necessary cause of paratuberculosis, but not sufficient in itself to cause the development of disease. The progression of paratuberculosis and the development of clinical signs, although innately linked with the process of becoming infected with *M a paratuberculosis,* are mostly controlled by immunocompromising factors, such as micronutrient deficiency or imbalance, rather than those predisposing to the acquisition of the organisms. Although experimentally it has been shown that the size of the inoculating dose has an effect on clinical expression of paratuberculosis,[68] this effect may be relatively unimportant in natural infections where the size of the dose is likely to be some orders of magnitude less than in most experimental studies where it is otherwise difficult to establish infection.[69] By introducing hygiene control measures designed to reduce infectious contacts, it would be expected the incidence of clinical expression will be below the maximum permissible by the environment had not these measures been applied. Such measures will not eliminate infection or reduce the potential maximum mortality rate.[70] The prevalence of faecal shedders, stock density, method of grazing and survival of the organism are likely to be important epidemiological factors,[68] but only in determining the time taken for the incidence of clinical disease to stabilise at the maximum incidence permissible by nutritional and other important environmental factors.[70]

Topics for future research

Concurrent studies of macrophage biology, trace element effects and interactions with phagosomes infected with *M a paratuberculosis* and other mycobacteria, and apoptotic processes need to be furthered or instigated. These efforts should allow improvements in our basic understanding of how micronutrient nutrition affects the outcome of infection with mycobacteria at the cellular level. Studies of periparturient ruminants might also confirm the occurrence of severe disturbances to mineral balance, particularly in dairy cattle, at this time. Mycobacterial dormancy, although receiving considerable attention in relation to human mycobacterioses, also requires further investigation to fully elucidate the role of this state in the pathogenesis and epidemiology of animal diseases. Such studies should provide useful supporting information to field investigations of the effects of acidic soils and dietary micronutrient imbalance on the outcome of infection with pathogenic mycobacteria.

Conclusions

Taken together these pieces of information provide additional supporting evidence to experimental and epidemiological studies and field observations that implicate excesses of iron and deficiencies of copper and selenium and possibly other elements such as zinc, manganese, and calcium in the clinical expression of mycobacterial diseases. For optimal physiological and immunological function the composition of the mineral component of the diet must be sufficient and balanced. These optimal dietary conditions should allow the host to eradicate the infecting bacilli or at least promote the development of a dormant infectious state. Our lack of detailed knowledge of the actual effect of these nutritional factors suggests that more research directed at understanding the role of these elements and their actual involvement in paratuberculosis and other mycobacterioses is required.

Postscript

The current paradigm suggests that it is the spread of infection from animal to animal or region to region which needs to be prevented or limited. This thinking stems from the adoption of Koch's Postulates, whereby we believe that if a pathogen is present in tissues it must be the cause of disease. However, in relation to *M a paratuberculosis* and the other pathogenic mycobacteria, this thinking is flawed. These organisms will lie dormant in tissues without causing any significant pathology and in this condition our ability to diagnose such infections is extremely limited.

The ability of pathogenic mycobacteria to lie dormant until the host is in some way immunocompromised is likely to be one of the key survival strategies of this group of potential pathogens. This is well recognised in human medicine, especially with respect to tuberculosis and infections with *M avium*, but has not been widely embraced in veterinary circles.

If, as suggested, the organism is only pathogenic in particular environments then it follows that the infection may be extremely widespread and that there is little use in attempting to prevent naive animals from becoming infected. What then becomes important is preventing the development of clinical disease in animals carrying these potential pathogens in a subclinical or dormant state.

I will have achieved my objective in writing these papers if I have helped to set the stage for a more holistic consideration of the factors that control the expression of paratuberculosis and may have convinced some of you that the pursuit of ensuring that animals are not exposed to infection with *M a paratuberculosis* is not necessarily the most important area of endeavour. Through following this line of thought there is considerable potential for improved progress in controlling paratuberculosis and in creating substantial benefits for farmers, animal health and welfare, and the environment.

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