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Age at which dairy cattle become Mycobacterium avium subsp. paratuberculosis faecal culture positive
Preventive Veterinary Medicine, 97, 29-36

Age at which cattle become faecal culture positive for Mycobacterium avium subsp. paratuberculosis (Map) can be used as a proxy parameter for age at onset of faecal shedding, which is an important parameter in the control of Map in cattle herds. To investigate the age at becoming faecal culture positive, survival analysis methods were applied. The analyses were carried out on asynchronous interval censored data of faecal culture results of samples collected from 18,979 female Holstein-Frisian cattle in 353 Dutch herds between 1996 and 2002. The data were analysed with a Weibull proportional hazards model. The results indicate that the distribution of age at onset of faecal shedding in Holstein-Frisian dairy cattle in infected herds is associated with the within-herd prevalence. In higher classes of apparent prevalence, cattle started to shed Map at younger age on average. In herds with an apparent prevalence <0.05, 0.05-0.1, 0.1-0.2 and >= 0.2, the proportion (95% CI) of cattle with onset of faecal shedding before 2 years of age was estimated at 1% (0.5%; 2%), 4% (3%; 5%), 8% (5%; 10%) and 20% (11%; 32%), respectively. This study indicates that a considerable proportion of young stock is shedding Map, especially in high prevalence herds. Therefore, infectious young stock should be a major concern in the control of paratuberculosis. (C) 2010 Elsevier B.V. All rights reserved

863 Rosenfeld, G., Bressler, B. (2010)
Mycobacterium avium paratuberculosis and the etiology of Crohn's disease: A review of the controversy from the clinician's perspective
Canadian Journal of Gastroenterology, 24, 619-624

Mycobacterium avium paratuberculosis (MAP) is an obligate intracellular organism that has frequently been associated with Crohn's disease (CD). Because CD is a chronic inflammatory condition, many researchers have speculated that an infectious agent must be the cause of CD. MAP has often been proposed to be one such agent; however, despite considerable research, the evidence remains inconclusive. Higher levels of MAP have been found in the tissues and blood of CD patients than in controls, forming the foundation for much of the research into the role of MAP in CD and the primary argument in support of a causative role for MAP in CD. MAP is a slow-growing and fastidious organism that is difficult to grow in culture and, therefore, challenging to detect in patients. As a result, there has been variability in the results of studies attempting to detect the presence of MAP in CD patients, and considerable controversy over whether this organism has a causative role in the etiology of CD. Two main hypotheses exist with respect to the role of MAP in CD. The first is that MAP is a principal cause of CD, while the second is that MAP is more prevalent because of the immune dysfunction seen in CD but does not play a causative role. Clinicians are often faced with questions regarding the role of this organism and the need to treat it. The present article attempts to provide an overview of the controversy including the nature of the mycobacterium, the difficulty in detecting it, the use of antimycobacterial agents to treat it and the effect of immunosuppressive agents - all from a clinician's perspective. Although the role of MAP in CD remains controversial and an area of considerable research, it is currently only of academic
interest because there is no clinically useful test to identify the presence of the organism, and no evidence to support the use of antibiotics to eradicate it for the treatment of CD

**Seroprevalence of Mycobacterium avium subspecies paratuberculosis in cows in Umbria, Italy**
Veterinary Record, 167, 577-578

Abstract not available.

**Research on Mycobacterium avium during the period 1995 to 2009**
Veterinarni Medicina, 55, 473-482

Papers on Mycobacterium avium, published between 1995 and 2009 that are indexed in the databases Web of Science (R) (Thomson Reuters) and PubMed (U.S. National Library of Medicine) were analysed and 3377 papers, published by 11 197 authors from 2630 institution and 75 countries were compared. Mycobacterium avium is represented by four subspecies (M. avium subsp. avium, M. avium subsp. silvaticum, M. avium subsp. hominissuis, and M. avium subsp. paratuberculosis). Mycobacteria play an important role as human and animal pathogens and represent a potential risk to consumers as food and environmental pathogens and immunomodulators

**Serum Biochemistry, Serology, and Parasitology of Boreal Caribou (Rangifer Tarandus Caribou) in the Northwest Territories, Canada**
Journal of Wildlife Diseases, 46, 1096-1107

Boreal caribou (Rangifer tarandus caribou) are an ecologically and culturally important wildlife species and now range almost exclusively in the boreal forests of Canada, including the Northwest Territories, northern Alberta, and British Columbia. Boreal caribou are threatened throughout their Canadian range because of direct and indirect natural and anthropogenic factors. In the Northwest Territories, however, they have a continuous range that overall has not yet been subjected to the same degree of anthropogenic habitat fragmentation and degradation that has occurred elsewhere in Canada. To monitor the health of boreal caribou populations and individuals, we collected blood from 104 adult, female boreal caribou captured between March 2003 and February 2006 and measured serum biochemical parameters. Serum creatinine was higher in pregnant than in nonpregnant caribou. Several biochemical parameters differed among years, but they tended to be similar to those reported for reindeer. Serum antibodies were found to an alphaherpesvirus, Toxoplasma gondii, and to the Mycobacterium avium subspecies paratuberculosis in 37.5, 2.9, and 1.3% of boreal caribou, respectively. Fecal samples were collected from 149 boreal caribou, and Cryptosporidium sp. oocysts, Giardia sp. cysts, trichostrongyle ova, dorsal-spined nematode larvae, cestode ova, and Eimeria sp. were found. Trypanosoma sp. was detected in the blood of 72.1% of boreal caribou. Eimeria sp., Cryptosporidium sp., and Giardia sp. have not been previously reported in boreal caribou

**Consecutive Excretion of Mycobacterium avium Subspecies paratuberculosis in Semen of a Breeding Bull Compared to the Distribution in Feces, Tissue and Blood by IS900 and F57 Quantitative Real-Time PCR and Culture Examinations**
Journal of Veterinary Medical Science, 72, 1283-1288

Paratuberculosis (Johne's disease) has emerged as one of the most important diseases in cattle. The role of infected bull semen in the spread of infection remains unclear, as the correlation between the amount of excreted Mycobacterium avium subsp. paratuberculosis (semen and feces) and the infection load (blood and tissues) has not been defined. The aim of the present study was to study by culture, and a quantitative real-time polymerase chain
reaction, the presence of bacteria in consecutive semen, blood, and fecal samples collected from one infected Piedmont breeding bull during a 380-day period. Five out of seven blood samples and all nine semen samples were positive in the real-time quantitative polymerase chain reaction with 10(1) to 10(2) and 10(2) to 10(4) copies of IS900/F57 per ml, respectively. In all, there were 9 fecal culture positive samples with too numerous to count colony forming units and positive real-time quantitative polymerase chain reactions ranging from 105 to 107 copies of IS900/F57. After the bull was euthanized, Mycobacterium avium subsp. paratuberculosis was cultured from various parts of the small and large intestines, liver tissue and lymph nodes and from the epididymis and vesicular glands. The results demonstrate a wide extraintestinal distribution of the bacterium and that breeding bulls should be considered a source of paratuberculosis infection due to their contact with other breeding bulls and a high number of heifers and cows through the natural mating process.


We report the resequencing and revised annotation of the Mycobacterium avium subsp. paratuberculosis K10 genome. A total of 90 single-nucleotide errors and a 51-bp indel in the original K10 genome were corrected, and the whole genome annotation was revised. Correction of these sequencing errors resulted in 28 frameshift alterations. The amended genome sequence is accessible via the supplemental section of study SRR0600191 in the NCBI Sequence Read Archive and will serve as a valuable reference genome for future studies.


To determine antibacterial activity of capuramycin analogues SQ997, SQ922, SQ641 and RKS2244 against several non-tuberculous mycobacteria (NTM). In vitro antibiotic activities, i.e. MIC, MBC, rate of killing and synergistic interaction with other antibiotics, were evaluated. SQ641 was the most active compound against all the NTM species studied. The MIC of SQ641 was < 0.06-4 mg/L for Mycobacterium avium complex (MAC; n = 20), 0.125-2 mg/L for M. avium paratuberculosis (MAP; n = 9), 0.125-2 mg/L for Mycobacterium kansasii (MKN; n = 2), 0.25-1 mg/L for Mycobacterium abscessus (MAB; n = 11), 4 mg/L for Mycobacterium smegmatis (MSMG; n = 1), and 1 and 8 mg/L for Mycobacterium ulcerans (MUL; n = 1), by microdilution and agar dilution methods, respectively. SQ641 was bactericidal against NTM, with an MBC/MIC ratio of 1 to 32, and killed all mycobacteria faster than positive control drugs for each strain. In chequerboard titrations, SQ641 was synergistic with ethambutol against both MAC and MSMG, and was synergistic with streptomycin and rifabutin against MAB. In vitro, SQ641 was the most potent of the capuramycin analogues against all NTM tested, both laboratory and clinical strains.


The use of enzyme-linked immunosorbent assays (ELISAs) is recommended for Johne’s disease (JD) control in dairy herds. In 2006, we developed a novel ELISA test for JD, named EVELISA (ELISA using ethanol extract of Mycobacterium avium subsp. paratuberculosis), which showed higher sensitivity than commercial ELISA tests. To further investigate the performance of EVELISA, we obtained 38 serum samples from cattle in a JD-free herd with
suspected cases of serological false-positive reactions. When these samples were tested using the EVELISA and a commercial ELISA test, more than 70% of the samples were falsely identified as JD positive. Antibodies in the serum samples reacted strongly with antigens of various environmental mycobacteria, suggesting the presence of cross-reactive antibodies in the samples. The possible cross reactions in the EVELISA were inhibited markedly by the use of Mycobacterium phlei antigens for antibody absorption. When these samples were tested, 8 samples were classified as positive for JD by the EVELISA with the antibody absorption, whereas 27 samples were classified as positive for JD by the commercial ELISA. For an estimation of tentative sensitivity and specificity, the ELISA tests were performed on 38 serum samples from JD-negative herds with no suspected cases of serological false-positive reaction and 68 samples from cattle diagnosed as positive for M. avium subsp. paratuberculosis infection by fecal culture test. Sensitivity and specificity of the EVELISA with preabsorption of serum with M. phlei (“ethanol vortex absorbed-ELISA” or EVA-ELISA) were estimated to be 97.1% and 100%, respectively, whereas those of the commercial ELISA were 48.5% and 97.4%, respectively. Further, in 85 fecal culture-negative cattle in JD-positive herds, higher sensitivity of the EVA-ELISA than the commercial ELISA was demonstrated by a Bayesian analysis. This study indicates that the EVA-ELISA may form a basis for a sensitive diagnostic test with a higher level of specificity than that of the current commercial ELISA test.

Iron-sparing Response of Mycobacterium avium subsp paratuberculosis is strain dependent
BMC Microbiology, 10, Article Number: 268-Published: OCT 22 2010

Background: Two genotypically and microbiologically distinct strains of Mycobacterium avium subsp. paratuberculosis (MAP) exist - S and C MAP strains that primarily infect sheep and cattle, respectively. Concentration of iron in the cultivation medium has been suggested as one contributing factor for the observed microbiologic differences. We recently demonstrated that S strains have defective iron storage systems, leading us to propose that these strains might experience iron toxicity when excess iron is provided in the medium. To test this hypothesis, we carried out transcriptional and proteomic profiling of these MAP strains under iron-replete or -deplete conditions. Results: We first complemented M. smegmatis Delta ideR with IdeR of C MAP or that derived from S MAP and compared their transcription profiles using M. smegmatis mc(2)155 microarrays. In the presence of iron, sIdoR repressed expression of bfrA and MAP2073c, a ferritin domain containing protein suggesting that transcriptional control of iron storage may be defective in S strain. We next performed transcriptional and proteomic profiling of the two strain types of MAP under iron-replete and -deplete conditions. Under iron-replete conditions, C strain upregulated iron storage (BfrA), virulence associated (Esx-5 and antigen85 complex), and ribosomal proteins. In striking contrast, S strain downregulated these proteins under iron-replete conditions. iTRAQ (isobaric tag for relative and absolute quantitation) based protein quantitation resulted in the identification of four unannotated proteins. Two of these were upregulated by a C MAP strain in response to iron supplementation. The iron-sparing response to iron limitation was unique to the C strain as evidenced by repression of non-essential iron utilization enzymes (aconitase and succinate dehydrogenase) and upregulation of proteins of essential function (iron transport, [Fe-S] cluster biogenesis and cell division). Conclusions: Taken together, our study revealed that C and S strains of MAP utilize divergent metabolic pathways to accommodate in vitro iron stress. The knowledge of the metabolic pathways these divergent responses play a role in are important to 1) advance our ability to culture the two different strains of MAP efficiently, 2) aid in diagnosis and control of Johne’s disease, and 3) advance our understanding of MAP virulence.

Maximizing Capture Efficiency and Specificity of Magnetic Separation for Mycobacterium avium subsp paratuberculosis Cells
Applied and Environmental Microbiology, 76, 7550-7558
In order to introduce specificity for Mycobacterium avium subsp. paratuberculosis prior to a phage amplification assay, various magnetic-separation approaches, involving either antibodies or peptides, were evaluated in terms of the efficiency of capture (expressed as a percentage) of M. avium subsp. paratuberculosis cells and the percentage of nonspecific binding by other Mycobacterium spp. A 50:50 mixture of MyOne Tosylactivated Dynabeads coated with the chemically synthesized M. avium subsp. paratuberculosis-specific peptides biotinylated aMp3 and biotinylated aMptD (i.e., peptide-mediated magnetic separation [PMS]) proved to be the best magnetic-separation approach for achieving 85 to 100% capture of M. avium subsp. paratuberculosis and minimal (< 1%) nonspecific recovery of other Mycobacterium spp. (particularly if beads were blocked with 1% skim milk before use) from broth samples containing 10(3) to 10(4) CFU/ml. When PMS was coupled with a recently optimized phage amplification assay and used to detect M. avium subsp. paratuberculosis in 50-ml volumes of spiked milk, the mean 50% limit of detection (LOD50) was 14.4 PFU/50 ml of milk (equivalent to 0.3 PFU/ml). This PMS-phage assay represents a novel, rapid method for the detection and enumeration of viable M. avium subsp. paratuberculosis organisms in milk, and potentially other sample matrices, with results available within 48 h.

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (477-485)

477 Cadwell, K. (2010)
Crohn's Disease Susceptibility Gene Interactions, a NOD to the Newcomer ATG16L1
Gastroenterology, 139, 1448-1450
Abstract not available.

Research on Mycobacterium avium during the period 1995 to 2009
Veterinarni Medicina, 55, 473-482
Papers on Mycobacterium avium, published between 1995 and 2009 that are indexed in the databases Web of Science (R) (Thomson Reuters) and PubMed (U.S. National Library of Medicine) were analysed and 3377 papers, published by 11 197 authors from 2630 institution and 75 countries were compared. Mycobacterium avium is represented by four subspecies (M. avium subsp. avium, M. avium subsp. silvaticum, M. avium subsp. hominissuis, and M. avium subsp. paratuberculosis). Mycobacteria play an important role as human and animal pathogens and represent a potential risk to consumers as food and environmental pathogens and immunomodulators.

Inhibitory effects of alprazolam on the development of acute experimental autoimmune encephalomyelitis in stressed rats
Pharmacology Biochemistry and Behavior, 97, 350-356
The progression and development of multiple sclerosis (MS) has long been hypothesized to be associated with stress. Benzodiazepines have been observed to reduce negative consequences of stress on the immune system in experimental and clinical models, but there are no data on their effects on MS, or experimental autoimmune encephalomyelitis (EAE), a model for human MS. We designed experiments conducted to ascertain whether alprazolam could modify the clinical, histological and neuroendocrine manifestations of acute EAE in Lewis rats exposed to a chronic auditory stressor. EAE was induced by injection of an emulsion of MBP and complete Freund's adjuvant containing Mycobacterium tuberculosis H37Ra. Stress application and treatment with drugs (placebo or alprazolam) were initiated 5 days before inoculation and continued daily for the duration of the experiment (days 14 or 34 postinoculation). Our results show significant increases in the severity of neurological signs, the histological lesions of the spinal cord (inflammation), and the corticosterone plasmatic levels in stressed rats compared to those non-stressed ones. Treatment with alprazolam...
reversed the adverse effects of stress. These findings could have clinical implications in patients suffering from MS treated with benzodiazepines, so besides the psychopharmacological properties of alprazolam against stress, it has beneficial consequences on EAE. (C) 2010 Elsevier Inc. All rights reserved

**Chronic yersiniosis due to defects in the TLR5 and NOD2 recognition pathways**
Netherlands Journal of Medicine, 68, 310-315

Infection with Yersinia enterocolitica leads to a self-limiting disease, but in a small number of cases a protracted course can develop. The host genetic factors contributing to the advancement of the disease to the chronic phase are not known. We describe a patient suffering from an abdominal inflammatory mass due to chronic yersiniosis. Functional assays revealed defects in the recognition of flagellin by Toll-like receptor 5 (TLR5) and of muramyl dipeptide by NOD2, leading to a defective inflammatory response to Yersinia enterocolitica. Genetic sequencing showed that the patient was compound heterozygous for five different mutations in TLR5, while being homozygous for the 3020insC NOD2 mutation. In conclusion, we describe a patient in whom specific defects in the TLR5 and NOD2 recognition pathways led to chronic yersiniosis.

**Eating the enemy in Crohn's disease An old theory revisited**
Journal of Crohn's & Colitis, 4, 377-383

Several old and new observations suggest the existence in Crohn's disease of a phagocytic disorder of macrophages related to impaired bactericidal activity of host cells or to the presence of invasive bacteria that have developed strategies to counteract macrophage killing. It was recently reported that disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. Secretion of proinflammatory cytokines by CD macrophages was impaired in response to E. coli or specific Toll-like receptor agonists. In addition, major advances in the etiology of Crohn's disease came from the existence of polymorphism in NOD2 and autophagy-related susceptibility genes (ATG16L1 and IRGM) in patients and from the identification of the presence of adherent-invasive E. coli (AIEC) colonizing the CD ileal mucosa and able to resist to macrophage killing. The role of impaired autophagy in Crohn's disease patients has been recently reinforced by the observation that the peptidoglycan receptor NOD2, in addition to sense intracellular bacteria, can induce autophagy by recruiting the critical autophagy protein ATG16L1 to the plasma membrane during bacterial internalization. Defects in autophagy might be the key element of the pathogenic pathway that lead to defective microbial killing, increased exposure to commensal and pathogenic intestinal bacteria and T cell activation. Defects in Paneth cells secreting lysozyme and antimicrobial peptides are observed in patients with ATG16L1 risk allele. Thus, the induction of autophagy or administration of preparations that mirrors the secretion of Paneth cells or both may be regarded as new therapeutic avenues for the treatment of Crohn's disease. (C) 2010 European Crohn's and Colitis Organisation. Published by Elsevier B.V. All rights reserved

**Nod1 and Nod2 Regulation of Inflammation in the Salmonella Colitis Model**
Infection and Immunity, 78, 5107-5115

The pattern recognition molecules Nod1 and Nod2 play important roles in intestinal homeostasis; however, how these proteins impact on the development of inflammation during bacterial colitis has not been examined. In the streptomycin-treated mouse model of Salmonella colitis, we found that mice deficient for both Nod1 and Nod2 had attenuated inflammatory pathology, reduced levels of inflammatory cytokines, and increased colonization of the mucosal tissue. Nod1 and Nod2 from both hematopoietic and nonhematopoietic...
sources contributed to the pathology, and all phenotypes were recapitulated in mice deficient for the signaling adaptor protein Rip2. However, the influence of Rip2 was strictly dependent on infection conditions that favored expression of the Salmonella pathogenicity island 2 (SPI-2) type III secretion system (TTSS), as Rip2 was dispensable for inflammation when mice were infected with bacteria grown under conditions that promoted expression of the SPI-1 TTSS. Thus, Nod1 and Nod2 can modulate inflammation and mediate efficient clearance of bacteria from the mucosal tissue during Salmonella colitis, but their role is dependent on the expression of the SPI-2 TTSS.

**Function of NOD-like receptors in immunity and disease**  
Current Opinion in Investigational Drugs, 11, 1246-1255

Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) are cytosolic pattern-recognition receptors that sense microbial invasion, cell stress and physiological perturbations, and elicit an inflammatory response to alert the system to the presence of danger. Most NLRs exert their functions by assembling inflammasomes that recruit and activate caspase-1, whereas a few engage the NF boxed times B and MAPK pathways. In the past few years, significant insights have been gained into the regulatory mechanisms of these innate immunity effectors and their role in health and disease that, notably, have led to direct therapeutic applications in the clinic. This review discusses the biology of NLRs, focusing on recent advances in the field that indicate a broader role for these proteins than had been previously anticipated, such as in priming systemic innate immunity, driving adaptive immunity, maintaining tissue homeostasis and inducing tissue repair following injury.

**Th1-driven immune reconstitution disease in Mycobacterium avium-infected mice**  
Blood, 116, 3485-3493

Following antiretroviral therapy, a significant proportion of HIV+ patients with mycobacterial coinfections develop a paradoxical, poorly understood inflammatory disease termed immune reconstitution inflammatory syndrome (IRIS). Here, we show that Mycobacterium avium-infected T cell-deficient mice injected with CD4 T cells also develop an immune reconstitution disease (IRD) manifesting as weight loss, impaired lung function, and rapid mortality. This form of IRD requires Ag recognition and interferon gamma production by the donor CD4 T cells and correlates with marked alterations in blood and tissue CD11b(+) myeloid cells. Interestingly, disease is associated with impaired, rather than augmented, T-cell expansion and function and is not strictly dependent on lymphopenia-induced T-cell proliferation. Instead, our findings suggest that mycobacterial-associated IRIS results from a heightened sensitivity of infected lymphopenic hosts to the detrimental effects of Ag-driven CD4 T-cell responses. (Blood. 2010;116(18):3485-3493)

**The protein Nod2: An innate receptor more complex than previously assumed**  
Biochemical Pharmacology, 80, 2021-2031

For almost 10 years, Nod2 has been known as a cytosolic innate receptor able to sense peptidoglycan from Gram-positive and -negative bacteria and to trigger RIP2- and NF-kappa B-mediated pro-inflammatory and antibacterial response. Mutations in the gene encoding Nod2 in humans have been associated with Crohn's disease (CD). Mechanisms by which Nod2 variants can lead to CD development are still under investigation. The most admitted hypothesis suggests that the impaired function of Nod2 variants in intestinal epithelial and phagocytic cells results in deficiencies in epithelial-barrier function which subsequently lead to increased bacterial invasion and inflammation at intestinal sites. Very recent results have just reinforced this hypothesis by demonstrating that Nod2 wild-type (unlike Nod2 variants) could mediate autophagy, allowing an efficient bacterial clearance and adaptive immune response. Other recent data have attributed new roles to Nod2. Indeed, Nod2 has been...
shown to activate antiviral innate immune responses involving IRF3-dependent IFN-beta production after viral ssRNA recognition through a RIP2-independent mechanism requiring the mitochondrial adaptor protein MAVS. Recently, Nod2 has been also shown to be exquisitely tuned to detect mycobacterial infections and mount a protective immunity against these pathogens. (C) 2010 Elsevier Inc. All rights reserved.