2012-06-03-088 Paratuberculosis databases updated (2012-06-03)
To: (08) Mycobacterial diseases; (23) Veterinary education; (27) Scientific information
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New publications in the PARATUBERCULOSIS database (1219-1223)

1219 Coussens, P.M., Sipkovsky, S., Murphy, B., Roussey, J., Colvin, C.J. (2012) Regulatory T cells in cattle and their potential role in bovine paratuberculosis
Comparative Immunology Microbiology and Infectious Diseases, 35, 233-239

The intracellular bacterium Mycobacterium avium subspecies paratuberculosis (MAP) causes Johne's disease in wild and domestic ruminants. Johne's disease presents as a chronic enteritis with severe inflammation of intestinal tissues, characterized by widespread infiltration of macrophages, the target cell of MAP. Clinical signs of Johne's disease are typically accompanied by a loss of peripheral CD4+ T cell responses to MAP antigens and an increase in anti-MAP serum IgG levels. Recently, it was proposed that regulatory T cells might develop over the lengthy course of subclinical MAP infection. In the past five years, significant progress in defining bovine regulatory T cells has been made. These studies grew out of observations that IL-10 is produced by PBMCs in response to MAP antigen stimulation and that neutralization of this IL-10 could enhance IFN-gamma production from MAP-antigen reactive effector T cells. Depletion studies revealed that MAP responsive cell populations producing IL-10 were largely CD4+ and CD25+, although monocytes have also been shown to produce IL-10 in response to MAP. In addition, evidence for a regulatory population of gamma delta T cells has also begun to accumulate. We summarize current thinking regarding regulatory T cells in MAP infection and provide data suggesting a potential link between regulatory T cells, bovine leukemia virus, and MAP. (C) 2012 Elsevier Ltd. All rights reserved

Small Ruminant Research, 104, 191-194

A goat population named Garfagnina, from north-west Tuscany (Italy), was studied in order to evaluate the health status in an estimated population of 2500 animals. Data were collected from 31 rural farms and 320 animals. For each farm an anamnesis was collected and a clinical examination of animals was performed; sera and faecal samples were collected and tested for brucellosis, paratuberculosis, caprine arthritis encephalitis virus (CAEV), tularemia and gastro intestinal parasites. All the animals tested were clinically healthy: a moderate ecto-parasitism (Ixodidae) was present without any sign of illness or scrape; a good faecal-score was recorded. Faecal sample flotation showed a moderate prevalence for Eimeria spp. oocysts (29.7%); gastrointestinal strongyles were always present. Eggs per gram (EPG) and oocysts per gram (OPG) counts were low (OPG/EPG < 100). According to Italian norms, all the animals tested were brucellosis free; serodiagnosis showed a 6.58% seroprevalence of CAEV, 5.26% of paratuberculosis, and 0.5% of tularemia. The sanitary conditions of the population confirmed the healthy status of the animals: sporadic positiveness seems to be correlated with the presence of wild ungulate in the same area and should be investigated further. (c) 2011 Elsevier B.V. All rights reserved

1221 Thompson-Crispi, K.A., Mallard, B.A. (2012) Type 1 and type 2 immune response profiles of commercial dairy cows in 4 regions across Canada
Canadian Journal of Veterinary Research-Revue Canadienne de Recherche Veterinaire, 76, 120-128

Diseases of dairy cattle have adverse implications for both the dairy industry and animal welfare. Understanding adaptive immune response profiles of cattle on a national scale will provide insight into the potential for improving health and decreasing disease. The objectives of the present study were to evaluate immune response phenotypes of Holstein cows outside the peripartum period and to determine if antibody isotype bias to putative type 1 and type 2 test antigens is maintained. The cows, housed on commercial farms in 4 key dairy regions across Canada, were immunized with test
antigens to measure their ability to mount cell-mediated immune responses (CMIR) and antibody-mediated immune responses (AMIR). Delayed-type hypersensitivity (DTH) was used as an indicator of CMIR and primary and secondary serum antibodies of the immunoglobulin (Ig) G1 and IgG2 isotypes were used to determine AMIR to the test antigens. Immune response phenotypes varied significantly among regions, herds, and cows. Cows in Alberta had significantly higher DTH responses and secondary responses to the type 2 test antigen than those in other regions. However, cows in Alberta had significantly lower primary antibody responses. It was found that Alberta had the lowest incidence of mastitis caused by Escherichia coli and Staphylococcus aureus compared with other regions. The IgG1/IgG2 antibody isotype ratio confirmed the nature of the test antigens. This was the first study to evaluate adaptive immune response profiles and disease incidence of dairy cows on a national scale and it therefore provides a glimpse of the current situation in Canada.

**Isolation of the cell wall-associated 34 kD antigen of paratuberculosis by electro elution**


*African Journal of Microbiology Research, 6*, 1694-1699

Mycobacterium avium subsp. paratuberculosis is the causative agent of Jhone's disease or paratuberculosis in ruminants. Effective control of paratuberculosis has been hampered by the lack of a specific antigen for diagnosis of paratuberculosis in early stages of infection. The 34 kD cell wall protein of MAP have been suggested as major species-specific immunodominant antigen in Jhone's disease, but because of special interactions between MAP cell wall proteins the purification of 34 kD protein for further studies is difficult. In present study, a method has been developed for extraction of 34 kD antigen from SDS-PAGE gels. Following separation of extracted cell wall proteins by polyacrylamide gels, proteins were stained with coomassie blue and 34 kD protein isolated using electroelution. Then, salts and SDS were removed by dialysis and 34 kD antigen was concentrated by poly ethylene Glycol 6 kD. Analysis of the system efficiency showed high protein recovery from SDS-PAGE gels. The mentioned antigen may be candidate with potential for vaccine development against MAP infection.

**In vitro effects of citrus oils against Mycobacterium tuberculosis and non-tuberculous Mycobacteria of clinical importance**


*Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes, 47*, 736-741

We evaluated the in vitro activity of citrus oils against Mycobacterium tuberculosis and other non-tuberculous Mycobacterium species. Citrus essential oils were tested against a variety of Mycobacterium species and strains using the BACTEC radiometric growth system. Cold pressed terpenelless Valencia oil (CPT) was further tested using the Wayne model of in vitro latency. Exposure of M. tuberculosis and M. bovis BCG to 0.025 % cold pressed terpenelless Valencia orange oil (CPT) resulted in a 3-log decrease in viable counts versus corresponding controls. Inhibition of various clinical isolates of the M. avium complex and M. abscessus ranged from 2.5 to 5.2-logs. Some species/strains were completely inhibited in the presence of CPT including one isolate each of the following: the M. avium complex, M. chelonae and M. avium subsp. paratuberculosis. CPT also inhibited the growth of BCG more than 99 % in an in vitro model of latency which mimics anaerobic dormancy thought to occur in vivo. The activity of CPT against drug-resistant strains of the M. avium complex and M. abscessus suggest that the mechanism of action for CPT is different than that of currently available drugs. Inhibition of latently adapted bacilli offers promise for treatment of latent infections of MTB. These results suggest that the antimycobacterial properties of CPT warrant further study to elucidate the specific mechanism of action and clarify the spectrum of activity.

**New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database** (689)


*Polymorphisms of NOD2 and the risk of tuberculosis: a validation study in the Chinese population*

*International Journal of Immunogenetics, 39*, 233-240

A genome-wide association study (GWAS) of leprosy reported four specific genetic polymorphisms of NOD2 that were associated with susceptibility to Mycobacterium leprae in China. Considering the role of NOD2 in innate immune defence, we performed a study in a Chinese population to determine...
whether the same SNPs of NOD2 that were associated with disease caused by M.leprae were also associated with disease caused by Mycobacterium tuberculosis. We performed a frequency-matched casecontrol study in 1043 patients with pulmonary tuberculosis and 808 unaffected controls. All subjects were >15 years old and were Han Chinese from Jiangsu Province. We extracted DNA from a blood sample from each study participant. SNPs of rs3135499, rs7194886, rs8057341 and rs9302752 in the NOD2 gene were genotyped using a TaqMan-based allelic discrimination system. Using all possible patients with tuberculosis as cases, no significant association was found between the four specific SNPs and the risk of tuberculosis. In a subgroup analysis restricted to cases with bacteriologically confirmed tuberculosis (sputum culture positive), the variant genotype of rs7194886 was significantly associated with an altered risk of tuberculosis. Compared with the CC genotype, individuals carrying the CT/TT genotype of rs7194886 had an increased risk [odds ratio (OR) 1.35, 95% confidence interval (CI) (1.05-1.72)]. The association was stronger among tobacco smokers and males. By haplotype analysis, rs9302752Crs7194886T was associated with an increased risk of bacteriologically confirmed tuberculosis (sputum culture positive) (P = 0.039), but it was not significant after correcting for multiple comparisons. In summary, genetic polymorphisms of the SNP rs7194886 in the NOD2 gene, which were discovered in the GWAS of leprosy, might also be associated with the pulmonary tuberculosis in the Chinese population.