2012-10-12-151 Paratuberculosis databases updated (2012-10-12)
To: (08) Mycobacterial diseases; (23) Veterinary education; (27) Scientific information

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Edited by the Reference Laboratory for Paratuberculosis and Avian Tuberculosis World Organization for Animal Health (OIE) and Biomedical Technology, Epidemiology and Food Safety Global Network harbouring in the Veterinary Research Institute, Brno, Czech Republic.
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New publications in the PARATUBERCULOSIS database (1300-1302)

Enhanced Priming of Adaptive Immunity by Mycobacterium smegmatis Mutants with High-Level Protein Secretion
Clinical and Vaccine Immunology, 19, 1416-1425

Mycobacteria have features that make them attractive as potential vaccine vectors. The nonpathogenic and rapidly growing Mycobacterium smegmatis can express both Mycobacterium tuberculosis antigens and heterologous antigens from other pathogens, and it has been used as a viable vector for the development of live vaccines. In order to further improve antigen-specific immunogenicity of M. smegmatis, we screened a random transposon mutant library for mutants displaying enhanced efficiency of protein secretion (“high secretors”) and isolated 61 mutants showing enhanced endogenic and transgenic protein secretion. Sequence analysis identified a total of 54 genes involved in optimal secretion of insert proteins, as well as multiple independent transposon insertions localized within the same genomic loci and operons. The majority of transposon insertions occurred in genes that have no known protein secretion function. These transposon mutants were shown to prime antigen-specific CD8(+) T cell responses better than the parental strain. Specifically, upon introducing the simian immunodeficiency virus (SIV) gag gene into these transposon mutant strains, we observed that they primed SIV Gag-specific CD8(+) T cell responses significantly better than the control prime immunization in a heterologous prime/boost regimen. Our results reveal a dependence on bacterial secretion of mycobacterial and foreign antigens for the induction of antigen-specific CD8(+) T cell responses. The data also suggest that these M. smegmatis transposon mutants could be used as novel live attenuated vaccine strains to express foreign antigens, such as those of human immunodeficiency virus type 1 (HIV-1), and induce strong antigen-specific T cell responses.

Journal of Food Protection, 75, 1562-1571

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The objectives of this study were to estimate the prevalence of Salmonella for individual, pooled, and composite fecal samples and to compare culture results from each sample type
for determining herd Salmonella infection status and identifying Salmonella serovar(s). During the U.S. Department of Agriculture National Animal Health Monitoring System Dairy 2007 study, data and samples were collected from dairy operations in 17 major dairy states. As part of the study, composite fecal samples (six per operation) were collected from cow areas, such as holding pens, alleyways, and lagoons, where manure accumulates. Fecal samples also were collected from individual cows (35 per operation), and fecal sample pools were created by combining samples from 5 cows (7 per operation). A total of 1,541 composite fecal samples were collected from 260 operations in 17 states, and 406 (26.3%) of these samples were culture positive for Salmonella. Among the 116 operations for which all three sample types were obtained, 41.4% (48 operations) were Salmonella culture positive based on individual samples, 39.7% (46 operations) were positive based on pooled samples, and 49.1% (57 operations) were positive based on composite fecal samples. Relative to individual samples, the sensitivity of composite fecal samples for determining herd infection status was 85.4% and the sensitivity of pooled fecal samples was 91.7%. On 33.6% of operations (39 of 116), Salmonella was cultured from all three fecal sample types (individual, pooled, and composite), and 20 (51.3%) of these operations had exactly the same serovar in all three sample types. Use of composite fecal samples is less costly and time-consuming than use of individual or pooled samples and provides similar results for detecting the presence and identifying serovars of Salmonella in dairy herds. Therefore, composite sampling may be an appropriate alternative to culture of individual samples when assessing Salmonella status in dairy herds.

Effects of paratuberculosis on Friesian cattle carcass weight and age at culling
Spanish Journal of Agricultural Research, 10, 662-670

Bovine paratuberculosis (PTB) causes major economic losses to dairy farmers because of decreased milk production, poor body condition, weight loss and early culling. The aim of this study was to evaluate the effect of Mycobacterium avium subspecies paratuberculosis (MAP) infection on carcass weight and age at slaughter in Friesian cattle. A total of 1,014 adult cows slaughtered at two local abattoirs in the Basque Country were included in this study. MAP infection was determined by different methods: indirect ELISA on blood samples, detection of MAP in tissues by culture and real time PCR (rtPCR), and histopathological examination. Serial and parallel interpretations of these methods were also considered for setting the upper and lower infection rates. MAP infection was confirmed by at least one test in 58.9% of animals. Most infected cows were detected by histopathology (46.9%) and rtPCR (29.6%). Overall estimates of carcass mean weight losses ranged from 3.7% for cases identified by the presence of microscopic specific PTB inflammatory lesions to 12.4% for cases with positive results in the paratuberculosis antibody ELISA test. Isolation of high bacterial loads in tissues and occurrence of diffuse granulomatous enteritis were associated with the highest weight loses, 22.2% and 26.0% respectively. The life expectancy of seropositive cows and those showing diffuse lesions was reduced by nearly one year compared to that of non-infected ones. Our results provide consistent evidences of PTB effect on the reduction of slaughter weight and lifespan of dairy cows, which could be considered as surrogates of clinical disease.

New publications in the CROHN’S DISEASE AND PARATUBERCULOSIS database (740-742)

Microbial Dysbiozsis in Pediatric Patients with Crohn’s Disease
Journal of Clinical Microbiology, 50, 3258-3266

Microbial dysbiozsis has been suggested to be involved in the pathogenesis of Crohn’s disease (CD); however, many studies of gut microbial communities have been confounded by environmental and patient-related factors. In this study, the microbial flora of fecal samples from 19 children newly diagnosed with CD and 21 age-matched controls were analyzed using high-throughput sequencing to determine differences in the microbial composition between
CD patients and controls. Analysis of the microbial composition of specific bacterial groups revealed that Firmicutes percentages were significantly lower in CD patients than in controls and that this was due largely to changes in the class Clostridia. Bacteroidetes and Proteobacteria percentages were higher and significantly higher in CD patients than in controls, respectively. Both the detection frequencies of Bacteroidetes and Firmicutes correlated (positively and negatively, respectively) with the calculated pediatric Crohn's disease activity index scores of patients. Upon further analysis, differences in the microbial compositions of patients with mild disease and moderate to severe disease were identified. Our findings indicate that a combination of different bacterial species or a dynamic interplay between individual species is important for disease and is consistent with the dysbiosis hypothesis of CD.

Inflammatory Bowel Diseases, 18, 1723-1734

Background: Ideal biomarkers are required to be developed for the diagnosis and prediction of the treatment of inflammatory bowel disease (IBD). We have reported that alteration of N-linked oligosaccharides of immunoglobulin (Ig) G is a novel diagnostic marker of IBD. Oligosaccharide alterations of IgA, however, have not been investigated in IBD patients.

Methods: N- and O-linked oligosaccharides of serum IgA purified from 32 patients with Crohn's disease (CD), 30 patients with ulcerative colitis (UC), and 30 healthy volunteers (HV) were analyzed with high-performance liquid chromatography and mass spectrometry. Enzymes related to oligosaccharide attachment were investigated. Results: N-linked oligosaccharides of IgA were not different between IBD and HV. In contrast, the number of N-acetylgalactosamines per hinge glycopeptide (GalNAc/HP) in the O-linked oligosaccharides of IgA was significantly decreased in patients with CD compared with UC and HV. GalNAc/HP had high sensitivity and specificity for discriminating between CD and HV based on receiver operating characteristic analysis. Lower GalNAc/HP was associated with more severe disease activity of CD. Changes in GalNAc/HP levels in 6 weeks after treatment with infliximab were associated with the clinical activity of CD at 30 weeks. GalNAc transferase expression of naive B cells and extent of GalNAc attachment in IgA were significantly decreased by interleukin-21 in vitro. Conclusions: The number of GalNAc attached in the IgA O-linked glycans of CD patients was significantly decreased, and strongly correlated with the clinical activity. Alterations of GalNAc attachment in IgA could be useful as a novel diagnostic and prognostic marker of CD. (Inflamm Bowel Dis 2012;)

European Journal of Dermatology, 22, 488-494

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Interleukin-17A (IL-17A) is a proinflammatory cytokine that plays an important role in fighting pathogens at mucosal interfaces, by summoning neutrophils and upregulating cytoplasmatic antimicrobial peptides. So far, the presence of IL-17A in leprosy has not been demonstrated. The expression of IL-17A and related cytokines (IL-6 and IL-23p19) was addressed through RNA extraction and cDNA quantitative amplification in macerated biopsies of active lesions of 48 leprosy patients and 20 fragments of normal skin of individuals. Blood levels of IL-17A, IL-23p19 and IL-6 were determined by ELISA. We found an abrogated mRNA IL-17A response in all biopsies of leprosy patients, as compared with controls. Circulating IL-17A and IL-23p19 were undetectable in both patients and controls, but IL-6 was higher in lepromatous patients. Although at low levels, IL-17A mRNA in lepromatous patients had an inverse linear correlation with bacillary burden. Low expression of IL-17A in patients may be a constitutive genetic
feature of leprosy patients or a circumstantial event induced by the local presence of the pathogen, as an escape mechanism