2013-09-29-073 Paratuberculosis databases updated (2013-09-28)

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New publications in the PARATUBERCULOSIS database (1507-1511)

1507 Pithua, P., Espejo, L.A., Godden, S.M., Wells, S.J.
Is an individual calving pen better than a group calving pen for preventing transmission of Mycobacterium avium subsp paratuberculosis in calves? Results from a field trial
Research in Veterinary Science, (2013) 95, 398-404

The objective of this study was to quantify the efficacy of using individual calving pens (ICP) from which manure was removed between successive calving compared with group calving pens (GCP) for limiting transmission of Mycobacterium avium subsp paratuberculosis (MAP) in Holstein calves. Every other pregnant cow in three Minnesota MAP endemic herds was assigned to calve in either the ICP or the GCP within 48-72 h prior to expected calving. Heifer calves born in the ICP were assigned to the intervention group (n = 238) while heifer calves born in the GCP were considered controls (n = 211). Calves were separated from their dams as soon as was possible once the calf was found. The intervention within the ICP relative to the GCP was the removal of fecal material in the ICP immediately after each birth. Upon enrollment in 2005, calves were monitored into adulthood. Of the original animals enrolled, 318 were tested for MAP at least once in 2007, 2009, or 2010 using serum ELISA (ICP, n = 165; GCP, n = 141) and bacterial culture of feces (ICP, n = 173; GCP, n = 145) tests. Cox regression analysis was performed to evaluate the time until MAP test positivity. Cows born in the ICP had a hazard ratio of 0.37 (95% CI = 0.34-0.4) for testing MAP serum ELISA positive, compared with cows born in GCP. Similarly, cows born in the ICP had a hazard ratio of 0.09 (95% CI = 0.06-0.14) for testing MAP fecal culture positive, compared with cows born in GCP. The Cox proportional-hazard assumption was violated in both models such that differences observed in the instantaneous hazards of MAP positive outcomes between groups (ICP vs. GCP) subsequently diminished overtime. These findings indicate that using ICP for calving delays exposure to MAP in calves and provides an effective strategy for reducing peripartum MAP transmission risks in herds attempting to limit the impact of paratuberculosis.

1508 Kleinlutzum, D., Weaver, G., Schley, D.
Within-group contact of cattle in dairy barns and the implications for disease transmission
Research in Veterinary Science, (2013) 95, 425-429

The prevention, control and reduction of livestock diseases require a good understanding of how the underlying causative agents are transmitted. On livestock premises the rate of spread is strongly determined by the contact, both direct and indirect, between infectious and susceptible individuals. Here we consider contact amongst barn-housed dairy cattle, one of the most important UK livestock sectors. A novel observational study of faecal spread indicates that the level of contact an individual animal can have with other herd members via this transmission pathway is very high (80 +/- 4% within sub-units). Additional observational studies indicate the possible level of direct physical contact an animal has with other group members (an approximate Poisson distribution with a mean rate of 14.4 distinct individuals per hour), and the potential for indirect transfer via inanimate objects by considering the proportion of the herd that touched a given gatepost in the milking parlour each day (43 +/- 6%). Results suggest that mixing may be considered homogeneous for certain pathogens, but that the spread of diseases transmitted along only specific routes requires the incorporation of within group contact structures.

1509 Liaskos, C., Spyrou, V., Roggenbuck, D., Athanasiou, L.V., Orfanidou, T., Mavropoulos, A., Reinhold, D., Rigopoulou, E.I., Amiridis, G.S., Billinis, C., Bogdanos, D.P.
Crohn's disease-specific pancreatic autoantibodies are specifically present in ruminants with paratuberculosis: Implications for the pathogenesis of the human disease
Autoimmunity, (2013) 46, 388-394

Mycobacterium avium subspecies paratuberculosis (MAP) induces paratuberculosis (ptb) in ruminants and has clinical and histological features resebling Crohn's disease (CD). Pancreatic autoantibodies (PAB) targeting glycoprotein 2 (GP2) are specifically found in CD, but it is currently unknown whether these autoantibodies can be found in ruminants with ptb. IgG anti-MAP and anti-GP2 antibodies were tested by ELISA in 286 ruminants (212 sheep and 74 cattle). PAB testing was performed by indirect immunofluorescence (IF) using anti-sheep or anti-cattle specific antisera. PCR analysis confirmed the presence of MAP in anti-MAP positive samples. Anti-GP2 antibodies were more prevalent in anti-MAP antibody positive (26.9%) than in anti-MAP negative ruminants (8.7%, $p<0.001$). Anti-GP2 antibodies were found in 16/70 (22.9%) anti-MAP positive sheep compared to 10/142 (7%, $p=0.001$) anti-MAP antibody negative and in anti-MAP positive cattle than in negative counterparts (5/8 versus 8/66, $p=0.003$). Absorbance values for anti-GP2 antibodies were higher in cattle than in sheep (mean 21 AU/mL +/- 25.45D versus 12.2 AU/mL +/- 23 SD, $p<0.001$). There was no correlation between anti-GP2 and anti-MAP antibody concentrations. Anti-GP2 antibodies persisted up to 1/1000 and showed the characteristic IIF pancreatic pattern seen by anti-GP2 antibody positive CD samples. This is the first study to demonstrate the presence of CD-specific GP2-reactive pancreatic autoantibodies in MAP-infected ruminants. Our data suggest that CD and ptb are characterised by an antigen-driven loss of immunological tolerance to GP2, implying commonalities in the immunopathogenesis of the human and ruminant inflammatory bowel disorder.

1510 Schillinger, S., Bridger, P.S., Bulun, H., Fischer, M., Akineden, O., Seeger, T., Barth, S., Henrich, M., Doll, K., Bulte, M., Menge, C., Bauerfeind, R.
Flow Cytometric Detection of Mycobacterium avium subsp paratuberculosis-Specific Antibodies in Experimentally Infected and Naturally Exposed Calves
Clinical and Vaccine Immunology, (2013) 20, 1457-1465

A desirable test to diagnose infections with Mycobacterium avium subsp. paratuberculosis facilitates identification of infected cattle prior to the state of M. avium subsp. paratuberculosis shedding. This study aimed at adjusting a flow cytometry (FC)-based assay, using intact M. avium subsp. paratuberculosis bacteria as the antigen, for diagnosis of M. avium subsp. paratuberculosis infections in calves. Serum samples were collected from experimentally infected (n = 12) and naturally exposed (n = 32) calves. Samples from five calves from positive dams were analyzed to determine the dynamics of maternal antibodies. Samples from adult cattle with defined infection status served as the standard (18 M. avium subsp. paratuberculosis shedders, 22 M. avium subsp. paratuberculosis free). After preadsorption with Mycobacterium phlei, sera were incubated with M. avium subsp. paratuberculosis and M. avium subsp. avium bacterial suspensions, respectively, followed by the separate detection of bovine IgG, IgG1, IgG2, and IgM attached to the bacterial surface. M. avium subsp. paratuberculosis-specific sample/positive (S/P) ratios were compared to enzyme-linked immunosorbent assay (ELISA) S/P ratios. In adult cattle, the FC assay for IgG1 had a sensitivity of 78% at a specificity of 100%. Maternally acquired antibodies could be detected in calves up to 121 days of life. While all but two sera taken at day 100 +/- 10 postnatum from naturally exposed calves tested negative, elevated S/P ratios (IgG and IgG1) became detectable from 44 and 46 weeks postinoculation onwards in two calves infected experimentally. Even with the optimized FC assay, M. avium subsp. paratuberculosis-specific antibodies can only occasionally be detected in infected calves less than 12 months of age. The failure to detect such antibodies apparently reflects the distinct immunobiology of M. avium subsp. paratuberculosis infections rather than methodological constraints.

1511 Cha, S.B., Yoo, A., Park, H.T., Sung, K.Y., Shin, M.K., Yoo, H.S.
Analysis of Transcriptional Profiles to Discover Biomarker Candidates in Mycobacterium avium subsp paratuberculosis-Infected Macrophages, RAW 264.7
Journal of Microbiology and Biotechnology, (2013) 23, 1167-1175

Paratuberculosis (PTB) or Johne's disease is one of the most serious chronic debilitating diseases of ruminants worldwide that is caused by Mycobacterium avium subsp. paratuberculosis (MAP). MAP is

a slow-growing bacterium that has very long latent periods, resulting in difficulties in diagnosing and controlling the disease, especially regarding the diagnosis of fecal shedders of MAP without any clinical signs. Based on this situation, attempts were made to identify biomarkers that show early responses to MAP infection in a macrophage cell line, RAW 264.7. In response to the infection with the bacterium, a lot of genes were turned on and/or off in the cells. Of the altered genes, three different categories were identified based on the time-dependent gene expression patterns. Those genes were considered as possible candidates for biomarkers of MAP infection after confirmation by quantitative RT-PCR analysis. To the best of our knowledge, this is the first attempt at discovering the host transcriptomic biomarkers of PTB, although further investigation will be required to determine whether these biomarker candidates are associated within the natural host.

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (839-845)

839 Liaskos, C., Spyrou, V., Roggenbuck, D., Athanasiou, L.V., Orfanidou, T., Mavropoulos, A., Reinhold, D., Rigopoulou, E.I., Amiridis, G.S., Billinis, C., Bogdanos, D.P.
Crohn's disease-specific pancreatic autoantibodies are specifically present in ruminants with paratuberculosis: Implications for the pathogenesis of the human disease
Autoimmunity, (2013) 46, 388-394

Mycobacterium avium subspecies paratuberculosis (MAP) induces paratuberculosis (ptb) in ruminants and has clinical and histological features resebling Crohn's disease (CD). Pancreatic autoantibodies (PAB) targeting glycoprotein 2 (GP2) are specifically found in CD, but it is currently unknown whether these autoantibodies can be found in ruminants with ptb. IgG anti-MAP and anti-GP2 antibodies were tested by ELISA in 286 ruminants (212 sheep and 74 cattle). PAB testing was performed by indirect immunofluorescence (IF) using anti-sheep or anti-cattle specific antisera. PCR analysis confirmed the presence of MAP in anti-MAP positive samples. Anti-GP2 antibodies were more prevalent in anti-MAP antibody positive (26.9%) than in anti-MAP negative ruminants (8.7%, $p<0.001$). Anti-GP2 antibodies were found in 16/70 (22.9%) anti-MAP positive sheep compared to 10/142 (7%, $p=0.001$) anti-MAP antibody negative and in anti-MAP positive cattle than in negative counterparts (5/8 versus 8/66, $p=0.003$). Absorbance values for anti-GP2 antibodies were higher in cattle than in sheep (mean 21 AU/mL +/- 25.45D versus 12.2 AU/mL +/- 23 SD, $p<0.001$). There was no correlation between anti-GP2 and anti-MAP antibody concentrations. Anti-GP2 antibodies persisted up to 1/1000 and showed the characteristic IIF pancreatic pattern seen by anti-GP2 antibody positive CD samples. This is the first study to demonstrate the presence of CD-specific GP2-reactive pancreatic autoantibodies in MAP-infected ruminants. Our data suggest that CD and ptb are characterised by an antigen-driven loss of immunological tolerance to GP2, implying commonalities in the immunopathogenesis of the human and ruminant inflammatory bowel disorder.

840 Deshpande, N.P., Kaakoush, N.O., Wilkins, M.R., Mitchell, H.M.
Comparative genomics of Campylobacter concisus isolates reveals genetic diversity and provides insights into disease association
Bmc Genomics, (2013) 14, Article Number: 585 DOI: 10.1186/1471-2164-14-585 Published: AUG 28 2013

Background: In spite of its association with gastroenteritis and inflammatory bowel diseases, the isolation of Campylobacter concisus from both diseased and healthy individuals has led to controversy regarding its role as an intestinal pathogen. One proposed reason for this is the presence of high genetic diversity among the genomes of C. concisus strains. Results: In this study the genomes of six C. concisus strains were sequenced, assembled and annotated including two strains isolated from Crohn's disease patients (UNSW2 and UNSW3), three from gastroenteritis patients (UNSW1, UNSWCS and ATCC 51562) and one from a healthy individual (ATCC 51561). The genomes of C. concisus BAA-1457 and UNSWCD, available from NCBI, were included in subsequent comparative genomic analyses. The Pan and Core genomes for the sequenced C. concisus strains consisted of 3254 and 1556 protein coding genes, respectively. Conclusion: Genes were identified with specific

conservation in C. concisus strains grouped by phenotypes such as invasiveness, adherence, motility and diseased states. Phylogenetic trees based on ribosomal RNA sequences and concatenated host-related pathways for the eight C. concisus strains were generated using the neighbor-joining method, of which the 16S rRNA gene and peptidoglycan biosynthesis grouped the C. concisus strains

according to their pathogenic phenotypes. Furthermore, 25 non-synonymous amino acid changes with 14 affecting functional domains, were identified within proteins of conserved host-related pathways, which had possible associations with the pathogenic potential of C. concisus strains. Finally, the genomes of the eight C. concisus strains were compared to the nine available genomes of the well-established pathogen Campylobacter jejuni, which identified several important differences in the respiration pathways of these two species. Our findings indicate that C. concisus strains are genetically diverse, and suggest the genomes of this bacterium contain respiration pathways and modifications in the peptidoglycan layer that may play an important role in its virulence.

841 Marcinek, P., Jha, A.N., Shinde, V., Sundaramoorthy, A., Rajkumar, R., Suryadevara, N.C., Neela, S.K., van Tong, H., Balachander, V., Valluri, V.L., Thangaraj, K., Velavan, T.P.
LRRK2 and RIPK2 Variants in the NOD 2-Mediated Signaling Pathway Are Associated with Susceptibility to Mycobacterium leprae in Indian Populations
Plos One, (2013) 8, Article Number: e73103 DOI: 10.1371/journal.pone.0073103 Published: AUG 28 2013

In recent years, genome wide association studies have discovered a large number of gene loci that play a functional role in innate and adaptive immune pathways associated with leprosy susceptibility. The immunological control of intracellular bacteria M. leprae is modulated by NOD2-mediated signaling of Th1 responses. In this study, we investigated 211 clinically classified leprosy patients and 230 ethnically matched controls in Indian population by genotyping four variants in NOD2 (rs9302752A/G), LRRK2 (rs1873613A/G), RIPK2 (rs40457A/G and rs42490G/A). The LRRK2 locus is associated with leprosy outcome. The LRRK2 rs1873613A minor allele and respective rs1873613AA genotypes were significantly associated with an increased risk whereas the LRRK2 rs1873613G major allele and rs1873613GG genotypes confer protection in paucibacillary and leprosy patients. The reconstructed GA haplotypes from RIPK2 rs40457A/G and rs42490G/A variants was observed to contribute towards increased risk whereas haplotypes AA was observed to confer protective role. Our results indicate that a possible shared mechanisms underlying the development of these two clinical forms of the disease as hypothesized. Our findings confirm and validates the role of gene variants involved in NOD2-mediated signalling pathways that play a role in immunological control of intracellular bacteria M. leprae.

842 Salem, M., Seidelin, J.B., Rogler, G., Nielsen, O.H.
Muramyl dipeptide responsive pathways in Crohn's disease: from NOD2 and beyond
Cellular and Molecular Life Sciences, (2013) 70, 3391- 3404

Crohn's disease (CD) is one of main disease entities under the umbrella term chronic inflammatory bowel disease. The etiology of CD involves alterations in genetic, microbiological, and immunological factors. This review is devoted to the role of the bacterial wall compound muramyl dipeptide (MDP) for the activation of inflammatory pathways involved in the pathogenesis of CD. The importance of this molecule is underscored by the fact that (1) MDP, which is found in most Gram-negative and -positive bacteria, is able to trigger several immunological responses in the intestinal system, and (2) that alterations in several mediators of the MDP response including-but not restricted to-nucleotide oligomerization domain 2 (NOD2) are associated with CD. The normalization of MDP signaling is one of several important factors that influence the intestinal inflammatory response, a fact which emphasizes the pathogenic importance of MDP signaling for the pathogenesis of CD. The important aspects of NOD2 and non-NOD2 mediated effects of MDP for the development of CD are highlighted, as well as how alterations in these pathways might translate into the development of new therapeutic strategies.

843 Liang, D.C., Zuo, A.J., Shao, H., Born, W.K., O'Brien, R.L., Kaplan, H.J., Sun, D.M.
IL-23 Receptor Expression on gamma delta T Cells Correlates with Their Enhancing or Suppressive Effects on Autoreactive T Cells in Experimental Autoimmune Uveitis
Journal of Immunology, (2013) 191, 1118-1125

We have previously reported that, depending on their activation status, mouse gamma delta T cells can either enhance or inhibit the activity of IL-17(+) autoreactive T cells in experimental autoimmune uveitis. In this study, we showed that gamma delta T cells in naive C57BL/6 (B6) mouse do not express the IL-23R, whereas in immunized mice, it is expressed on >50% of gamma delta T cells. In

vitro studies showed that IL-23R expression on gamma delta T cells is modulated by their state of activation, as weakly activated gamma delta T cells expressed the IL-23R, but highly activated gamma delta T cells did not. Functional studies showed that IL-23R(+) gamma delta T cells had the strongest suppressive effect on IL-17(+) autoreactive T cells, and that this effect was inhibited when the IL-23R was blocked by anti-IL-23R Ab or in the presence of excessive amounts of exogenous IL-23. We conclude that the balance between the enhancing and inhibitory effects of gamma delta T cells is regulated by their level of IL-23R expression. The expression of variable IL-23R levels allows gamma delta T cells to have different regulatory effects on adaptive immune responses, conceivably as a result of ab and gamma delta T cells competing for IL-23.

844 Cha, S.B., Yoo, A., Park, H.T., Sung, K.Y., Shin, M.K., Yoo, H.S.
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845 Zulfiqar, F., Hozo, I., Rangarajan, S., Mariuzza, R.A., Dziarski, R., Gupta, D.
Genetic Association of Peptidoglycan Recognition Protein Variants with Inflammatory Bowel Disease
Plos One, (2013) 8, Article Number: e67393 DOI: 10.1371/journal.pone.0067393 Published: JUN 19 2013

Inflammatory bowel disease (IBD) is a common disease, includes Crohn's disease (CD) and ulcerative colitis (UC), and is determined by altered gut bacterial populations and aberrant host immune response. Peptidoglycan recognition proteins (PGLYRP) are innate immunity bactericidal proteins expressed in the intestine. In mice, PGLYRPs modulate bacterial populations in the gut and sensitivity to experimentally induced UC. The role of PGLYRPs in humans with CD and/or UC has not been previously investigated. Here we tested the hypothesis that genetic variants in PGLYRP1, PGLYRP2, PGLYRP3 and PGLYRP4 genes associate with CD and/or UC and with gender and/or age of onset of disease in the patient population. We sequenced all PGLYRP exons in 372 CD patients, 77 UC patients, 265 population controls, 210 familial CD controls, and 24 familial UC controls, identified all polymorphisms in these populations, and analyzed the variants for significant association with CD and UC. We identified 16 polymorphisms in the four PGLYRP genes that significantly associated with CD, UC, and/or subgroups of patient populations. Of the 16, 5 significantly associated with both CD and UC, 6 with CD, and 5 with UC. 12 significant variants result in amino acid substitutions and based on structural modeling several of these missense variants may have structural and/or functional

consequences for PGLYRP proteins. Our data demonstrate that genetic variants in PGLYRP genes associate with CD and UC and may provide a novel insight into the mechanism of pathogenesis of IBD.

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