2013-07-11-048 Paratuberculosis databases updated (2013-07-11)
(04) Mycobacterial diseases; (12) Scientific Information, research and education

****

New publications in the PARATUBERCULOSIS database (1456-1462)

1456 Elze, J., Liebler-Tenorio, E., Ziller, M., Kohler, H.

Comparison of prevalence estimation of Mycobacterium avium subsp paratuberculosis infection by sampling slaughtered cattle with macroscopic lesions vs. systematic sampling
Epidemiology and Infection, (2013) 141, 1536-1544
The objective of this study was to identify the most reliable approach for prevalence estimation of Mycobacterium avium ssp. paratuberculosis (MAP) infection in clinically healthy slaughtered cattle. Sampling of macroscopically suspect tissue was compared to systematic sampling. Specimens of ileum, jejunum, mesenteric and caecal lymph nodes were examined for MAP infection using bacterial microscopy, culture, histopathology and immunohistochemistry. MAP was found most frequently in caecal lymph nodes, but sampling more tissues optimized the detection rate. Examination by culture was most efficient while combination with histopathology increased the detection rate slightly. MAP was detected in 49/50 animals with macroscopic lesions representing 1.35% of the slaughtered cattle examined. Of 150 systematically sampled macroscopically non-suspect cows, 28.7% were infected with MAP. This indicates that the majority of MAP-positive cattle are slaughtered without evidence of macroscopic lesions and before clinical signs occur. For reliable prevalence estimation of MAP infection in slaughtered cattle, systematic random sampling is essential.

1457 Sorge, U.S. , Kurnick, S., Sreevatsan, S.

Detection of Mycobacterium avium subspecies paratuberculosis in the saliva of dairy cows: A pilot study
Veterinary Microbiology, (2013) 164, 383-386
Johne's disease (JD) is a production limiting, intestinal disease of ruminants that is caused by Mycobacterium avium subsp. paratuberculosis (MAP). Transmission of MAP occurs predominately through feces, colostrum and milk. Since other intestinal bacteria can be found in saliva, it possible that saliva might serve as a previously overlooked route of MAP transmission. Therefore, the objective of this study was to investigate whether MAP is present in the saliva of cows. Methods were validated using MAP 1(10 spiked saliva samples of cows from a voluntary JD control program level 4 herd and applied to saliva and fecal samples of cows from a known infected herd. The matched pairs of saliva and feces were analyzed for MAP with PCR and culture. Fourteen of the twenty-six sampled cows were saliva positive by conventional PCR. The fecal samples of 10 and 6 cows were positive by realtime PCR and MAP culture, respectively. Overall there was a poor agreement between saliva and fecal PCR results for MAP (kappa 0.24). This is the first study that detected MAP in the saliva of cows. The finding needs further investigation to identify the source of MAP in saliva and to quantify the role of this newly identified route of MAP emission for the transmission of MAP infections on farm.

1458 Vazquez, P. , Garrido, J.M., Juste, R.A.

Specific Antibody and Interferon-Gamma Responses Associated with Immunopathological Forms of Bovine Paratuberculosis in Slaughtered Friesian Cattle
Plos One, (2013) 8, Article Number: e64568 DOI: 10.1371/journal.pone.0064568
Published: MAY 28 2013-Mycobacterium avium subsp. paratuberculosis (MAP) infection causes a chronic granulomatous inflammatory regional enteritis in ruminants. Cell-mediated immune responses are assumed to be protective and therefore, to be associated with its more delimited lesion types, while humoral responses are mainly associated with diffuse histopathological lesions. However, this duality of immune responses has been recently questioned. The aim of this study was to assess the relationship between both types of

immunological responses and the type and extension of intestinal lesions and the presence of MAP in bovine tissues. Standard histopathological examinations, two microbiological procedures (culture and real time PCR (rtPCR)), as well as MAP specific antibody and interferon gamma (IFN-gamma) release assays (IGRA) were performed on tissues and blood of 333 slaughtered Holstein-Friesian animals. Paratuberculous lesions were observed in 176 (52.9%) of the animals and overall MAP detection rates were estimated at 13.5% and 28.5% for tissue culture and rtPCR, respectively. Unlike the relatively constant non-specific IFN-gamma release, both the antibody levels and the specific IFN-gamma release significantly increased with tissue damage. Delimited immunopathological forms, which accounted for 93.2% of all forms, were mostly related to positive testing in the IGRA (38.4%) whereas diffuse ones (6.8%) were associated with antibody seropositivity (91.7%). However, since the frequency of positive immune responses in both tests increased as the lesions severity increased, polarization of Th1/Th2 responses was less prominent than expected. MAP was detected in the majority of ELISA-positive animals (culture+: 90%, rtPCR+: 85%) but the bacteria was only confirmed in the 36.1% of IGRA-positive animals by any of the two microbiological tests. In terms of diagnosis, the antibody test was a good indicator of advanced tissue damage (diffuse forms), but the IGRA did not associate well with more delimited forms or with MAP detection.

1459 Guffey, J.S., Payne, W., James, L., Qian, Z.Y.

Inactivation of Mycobacterium Smegmatis Following Exposure to 405-Nanometer Light from a Supra luminous Diode Array
Wounds-A Compendium of Clinical Research and Practice, (2013) 25, 131-135
Objective. To determine the potential for blue light (405 nm) to produce a bactericidal effect on Mycobacterium smegmatis. Additionally, the study sought to evaluate a series of doses in terms of their respective bactericidal capabilities. Background Data. The effect of blue light on Staphylococcus aureus has been studied and it was found that a bactericidal outcome can be obtained with low doses of blue light.(1) Methods. M. smegmatis was tested because of the recent appearance of the Mycobacterium family of organisms as a public health threat among persons receiving tattoos. The organism was treated in vitro with 405 nm light emitted from a supraluminous diode (SLD) array. Doses of 60 Jcm(-2), 90 Jcm(-2), 120 Jcm(-2), 150 Jcm(-2), 180 Jcm(-2), 215 Jcm(-2), and 250 Jcm(-2) were used. Colony counts were performed and compared to untreated controls using Student t tests and one-way ANOVA with Tukey post hoc analysis. Results. The results revealed statistically significant bactericidal effects of the blue light on M. smegmatis (F6, 28 = 50.518, P = 0.000). The treatment reduced the number of bacterial colonies at all doses, but 60 Jcm(-2) did not produce a statistically significant kill rate. All other doses produced a significant kill rate with 120 Jcm(-2), 150 Jcm(-2), and 215 Jcm(-2), demonstrating the most effective kill rates of 98.3%, 96.7%, and 100%, respectively. Conclusions. Appropriate doses of 405 nm light from an SLD array can kill M. smegmatis in vitro. A dose of at least 100 Jcm(-2) dose is needed for the most effective inactivation of the organism. The dose response for this organism to blue light is not linear. Some degree of effectiveness is lost at 180 Jcm(-2) and 250 Jcm(-2).

1460 Botsaris, G., Liapi, M., Kakogiannis, C., Dodd, C.E.R., Rees, C.E.D.

Detection of Mycobacterium avium subsp paratuberculosis in bulk tank milk by combined phage-PCR assay: Evidence that plaque number is a good predictor of MAP
International Journal of Food Microbiology, (2013) 164, 76-80
Conventional culture and a rapid phage-PCR method were used to detect Mycobacterium avium subsp. paratuberculosis (MAP) in bulk tank milk (BTM) samples. Only two of 225 samples (0.9%) were found to contain MAP by culture whereas 50 (22%) MAP-positive samples were identified using the phage-PCR assay, including both samples that were MAP-culture positive. Results using the phage-based method for independently tested duplicate samples indicated that the assay is very reproducible (r(2) = 0.897), especially when low levels of mycobacteria are present. A relationship was established between plaque number and the presence of MAP in a sample. A cut-off value was determined allowing identification of MAP-positive samples based on plaque number alone (90% sensitivity, 99% specificity;

area under the curve = 0.976). These results indicate that the assay is a robust method for screening BTM, providing results within 24 h. (C) 2013 Elsevier B.V. All rights reserved.

1461 Salgado, M. , Alfaro, M., Salazar, F., Troncoso, E., Mitchell, R.M., Ramirez, L., Naguil, A., Zamorano, P., Collins, M.T.

Effect of Soil Slope on the Appearance of Mycobacterium avium subsp paratuberculosis in Water Running off Grassland Soil after Application of Contaminated Slurry

Applied and Environmental Microbiology, (2013) 79, 3544-3552

The study assessed the effect of soil slope on Mycobacterium avium subsp. paratuberculosis transport into rainwater runoff from agricultural soil after application of M. avium subsp. paratuberculosis-contaminated slurry. Under field conditions, 24 plots of undisturbed loamy soil 1 by 2m(2) were placed on platforms. Twelve plots were used for water runoff: 6 plots at a 3% slope and 6 plots at a 15% slope. Half of the plots of each slope were treated with M. avium subsp. paratuberculosis-contaminated slurry, and half were not treated. Using the same experimental design, 12 plots were established for soil sampling on a monthly basis using the same spiked slurry application and soil slopes. Runoff following natural rainfall was collected and analyzed for M. avium subsp. paratuberculosis, coliforms, and turbidity. M. avium subsp. paratuberculosis was detected in runoff from all plots treated with contaminated slurry and one control plot. A higher slope (15%) increased the likelihood of M. avium subsp. paratuberculosis detection but did not affect the likelihood of finding coliforms. Daily rainfall increased the likelihood that runoff would have coliforms and the coliform concentration, but it decreased the M. avium subsp. paratuberculosis concentration in the runoff. When there was no runoff, rain was associated with increased M. avium subsp. paratuberculosis concentrations. Coliform counts in runoff were related to runoff turbidity. M. avium subsp. paratuberculosis presence/absence, however, was related to turbidity. Study duration decreased bacterial detection and concentration. These findings demonstrate the high likelihood that M. avium subsp. paratuberculosis in slurry spread on pastures will contaminate water runoff, particularly during seasons with high rainfall. M. avium subsp. paratuberculosis contamination of water has potential consequences for both animal and human health.

1462 Masala, S., Zedda, M.A., Cossu, D., Ripoli, C., Palermo, M., Sechi, L.A.

Zinc Transporter 8 and MAP3865c Homologous Epitopes are Recognized at T1D Onset in Sardinian Children

Plos One, (2013) 8, Article Number: e63371 DOI: 10.1371/journal.pone.0063371

Published: MAY 17 2013-Our group has recently demonstrated that Mycobacterium avium subspecies paratuberculosis (MAP) infection significantly associates with T1D in Sardinian adult patients. Due to the potential role played by MAP in T1D pathogenesis, it is relevant to better characterize the prevalence of anti-MAP antibodies (Abs) in the Sardinian population, studying newly diagnosed T1D children. Therefore, we investigated the seroreactivity against epitopes derived from the ZnT8 autoantigen involved in children at T1D onset and their homologous sequences of the MAP3865c protein. Moreover, sera from all individuals were tested for the presence of Abs against: the corresponding ZnT8 C-terminal region, the MAP specific protein MptD, the T1D autoantigen GAD65 and the T1D unrelated Acetylcholine Receptor. The novel MAP3865c(281-287) epitope emerges here as the major C-terminal epitope recognized. Intriguingly ZnT8(186-194) immunodominant peptide was cross-reactive with the homologous sequences MAP3865c(133-141), strengthening the hypothesis that MAP could be an environmental trigger of T1D through a molecular mimicry mechanism. All eight epitopes were recognized by circulating Abs in T1D children in comparison to healthy controls, suggesting that these Abs could be biomarkers of T1D. It would be relevant to investigate larger cohorts of children, followed over time, to elucidate whether Ab titers against these MAP/Znt8 epitopes wane after diagnosis.

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (813-814)

813 Spiering, R. , van der Zee, R., Wagenaar, J., Van Eden, W., Broere, F.

Mycobacterial and mouse HSP70 have immuno-modulatory effects on dendritic cells
Cell Stress & Chaperones, (2013) 18, 439-446
Previously, it has been shown that heat shock protein 70 (HSP70) can prevent inflammatory damage in experimental autoimmune disease models. Various possible underlying working mechanisms have been proposed. One possibility is that HSP70 induces a tolerogenic phenotype in dendritic cells (DCs) as a result of the direct interaction of the antigen with the DC. Tolerogenic DCs can induce antigen-specific regulatory T cells and dampen pathogenic T cell responses. We show that treatment of murine DCs with either mycobacterial (Mt) or mouse HSP70 and pulsed with the disease-inducing antigen induced suppression of proteoglycan-induced arthritis (PGIA), although mouse HSP70-treated DCs could ameliorate PGIA to a greater extent. In addition, while murine DCs treated with Mt- or mouse HSP70 had no significantly altered phenotype as compared to untreated DCs, HSP70-treated DCs pulsed with pOVA (ovalbumin peptide 323-339) induced a significantly increased production of IL-10 in pOVA-specific T cells. IL-10-producing T cells were earlier shown to be involved in Mt HSP70-induced suppression of PGIA. In conclusion, this study indicates that Mt- and mouse HSP70-treated BMDC can suppress PGIA via an IL-10-producing T cell-dependent manner.

814 Shenderov, K., Barber, D.L., Mayer-Barber, K.D., Gurcha, S.S., Jankovic, D., Feng, C.G., Oland, S., Hieny, S., Caspar, P., Yamasaki, S., Lin, X., Ting, J.P.Y., Trinchieri, G., Besra, G.S., Cerundolo, V., Sher, A.

Cord Factor and Peptidoglycan Recapitulate the Th17-Promoting Adjuvant Activity of Mycobacteria through Mincle/CARD9 Signaling and the Inflammasome
Journal of Immunology, (2013) 190, 5722-5730
Although adjuvants are critical vaccine components, their modes of action are poorly understood. In this study, we investigated the mechanisms by which the heat-killed mycobacteria in CFA promote Th17 CD4(+) T cell responses. We found that IL-17 secretion by CD4(+) T cells following CFA immunization requires MyD88 and IL-1 beta/IL-1R signaling. Through measurement of Ag-specific responses after adoptive transfer of OTII cells, we confirmed that MyD88-dependent signaling controls Th17 differentiation rather than simply production of IL-17. Additional experiments showed that CFA-induced Th17 differentiation involves IL-1 beta processing by the inflammasome, as mice lacking caspase-1, ASC, or NLRP3 exhibit partially defective responses after immunization. Biochemical fractionation studies further revealed that peptidoglycan is the major component of heat-killed mycobacteria responsible for inflammasome activation. By assaying Il lb transcripts in the injection site skin of CFA-immunized mice, we found that signaling through the adaptor molecule caspase activation and recruitment domain 9 (CARD9) plays a major role in triggering pro-IL-1 beta expression. Moreover, we demonstrated that recognition of the mycobacterial glycolipid trehalose dimycolate (cord factor) by the C-type lectin receptor mincle partially explains this CARD9 requirement. Importantly, purified peptidoglycan and cord factor administered in mineral oil synergized to recapitulate the Th17-promoting activity of CFA, and, as expected, this response was diminished in caspase-1 and CARD9-deficient mice. Taken together, these findings suggest a general strategy for the rational design of Th17-skewing adjuvants by combining agonists of the CARD9 pathway with inflammasome activators.

****