



2013-07-01-045 Paratuberculosis databases updated (2013-07-01)

A special service of the OIE World Organization for Animal Health [Reference Laboratory for Paratuberculosis](#), Brno, Czech Republic to registered members of the Biomedical Technology, Epidemiology and Food Safety network.

How to request [full papers](#) from PTB databases

CENTAUR GLOBAL NETWORK INFORMATION Vol. 17, 2013, issue 045 is distributed to:
(04) Mycobacterial diseases; (12) Scientific Information, research and education; .

New publications in the [PARATUBERCULOSIS database](#) (1443-1455)

1443 Kostoulas, P., Nielsen, S.S., Browne, W.J. , Leontides, L.

Sample size estimation to substantiate freedom from disease for clustered binary data with a specific risk profile

Epidemiology and Infection, (2013) 141, 1318-1327

Disease cases are often clustered within herds or generally groups that share common characteristics. Sample size formulae must adjust for the within-cluster correlation of the primary sampling units. Traditionally, the intra-cluster correlation coefficient (ICC), which is an average measure of the data heterogeneity, has been used to modify formulae for individual sample size estimation. However, subgroups of animals sharing common characteristics, may exhibit excessively less or more heterogeneity. Hence, sample size estimates based on the ICC may not achieve the desired precision and power when applied to these groups. We propose the use of the variance partition coefficient (VPC), which measures the clustering of infection/disease for individuals with a common risk profile. Sample size estimates are obtained separately for those groups that exhibit markedly different heterogeneity, thus, optimizing resource allocation. A VPC-based predictive simulation method for sample size estimation to substantiate freedom from disease is presented. To illustrate the benefits of the proposed approach we give two examples with the analysis of data from a risk factor study on Mycobacterium avium subsp. paratuberculosis infection, in Danish dairy cattle and a study on critical control points for Salmonella cross-contamination of pork, in Greek slaughterhouses.

1444 Wagner, J., Sim, W.H., Lee, K.J., Kirkwood, C.D.

Current knowledge and systematic review of viruses associated with Crohn's disease

Reviews in Medical Virology, (2013) 23, 145-171

The aetiology of Crohn's disease (CD) is currently unknown. A viral trigger was proposed more than 40 years ago and has been the focus of many investigations. We summarised the current literature surrounding the association between viruses and CD and conducted a systematic review of all studies investigating this association quantitatively. Studies were identified by searching for 13 specific virus names or the general term 'virus' and 'Crohn's disease' in search engines PubMed and OVID. A total of 1315 studies were identified, of which 78 studies had a laboratory result. Of the 78, 46 casecontrol studies met all the inclusion criteria for forest plot analysis. The most common viruses studied were EBV, CMV and measles virus (MV). Forest plot analysis for each virus was carried out (fitted using random effects) and identified evidence of an association between CD and CMV (risk ratio [RR] 1.602, 95% confidence interval [CI] 1.069 to 2.400) with some suggestion that EBV may also be associated with CD (RR 1.366, 95% CI 0.996 to 1.873). However, there was evidence of large heterogeneity in the results from the identified studies for EBV. There was little evidence of an association with CD for MV, human herpes virus 6, human herpes virus 8, human simplex virus, varicella-zoster virus, mumps virus, Rubella virus, rotavirus, norovirus and adenovirus. There is still some question around whether CD is associated with the presence of a currently known virus. Copyright (c) 2012 John Wiley & Sons, Ltd.



1445 Nalpas, N.C., Park, S.D.E., Magee, D.A., Taraktsoglou, M., Browne, J.A., Conlon, K.M., Rue-Albrecht, K., Killick, K.E., Hokamp, K., Lohan, A.J., Loftus, B.J., Gormley, E., Gordon, S.V., MacHugh, D.E.

Whole-transcriptome, high-throughput RNA sequence analysis of the bovine macrophage response to *Mycobacterium bovis* infection in vitro

Bmc Genomics, (2013) 14, Article Number: 230 DOI: 10.1186/1471-2164-14-230
Published: APR 8 2013-Background: *Mycobacterium bovis*, the causative agent of bovine tuberculosis, is an intracellular pathogen that can persist inside host macrophages during infection via a diverse range of mechanisms that subvert the host immune response. In the current study, we have analysed and compared the transcriptomes of *M. bovis*-infected monocyte-derived macrophages (MDM) purified from six Holstein-Friesian females with the transcriptomes of non-infected control MDM from the same animals over a 24 h period using strand-specific RNA sequencing (RNA-seq). In addition, we compare gene expression profiles generated using RNA-seq with those previously generated by us using the high-density Affymetrix (R) GeneChip (R) Bovine Genome Array platform from the same MDM-extracted RNA. Results: A mean of 7.2 million reads from each MDM sample mapped uniquely and unambiguously to single *Bos taurus* reference genome locations. Analysis of these mapped reads showed 2,584 genes (1,392 upregulated; 1,192 downregulated) and 757 putative natural antisense transcripts (558 upregulated; 119 downregulated) that were differentially expressed based on sense and antisense strand data, respectively (adjusted P-value \leq 0.05). Of the differentially expressed genes, 694 were common to both the sense and antisense data sets, with the direction of expression (i.e. up- or downregulation) positively correlated for 693 genes and negatively correlated for the remaining gene. Gene ontology analysis of the differentially expressed genes revealed an enrichment of immune, apoptotic and cell signalling genes. Notably, the number of differentially expressed genes identified from RNA-seq sense strand analysis was greater than the number of differentially expressed genes detected from microarray analysis (2,584 genes versus 2,015 genes). Furthermore, our data reveal a greater dynamic range in the detection and quantification of gene transcripts for RNA-seq compared to microarray technology. Conclusions: This study highlights the value of RNA-seq in identifying novel immunomodulatory mechanisms that underlie host-mycobacterial pathogen interactions during infection, including possible complex post-transcriptional regulation of host gene expression involving antisense RNA.

1446 Singh, K., Chandel, B.S., Chauhan, H.C., Dadawala, A., Singh, S.V., Singh, P.K.

Efficacy of 'indigenous vaccine' using native 'Indian bison type' genotype of *Mycobacterium avium* subspecies paratuberculosis for the control of clinical Johne's disease in an organized goat herd

Veterinary Research Communications, (2013) 37, 109-114
Therapeutic efficacy of a new 'Indigenous vaccine' prepared from native highly pathogenic 'Indian Bison Type' genotype of *Mycobacterium avium* subspecies paratuberculosis (MAP) of goat origin has been evaluated with respect to control of clinical Johne's disease in naturally infected Mehsana breed of goat in North Gujarat. Fifty goats from Sheep and Goats Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, were randomly divided into 2 groups viz., 'Vaccinated' (n = 35) and 'Control' (n = 15). After vaccination, goats were monitored for physical condition, morbidity, mortality, body weights, shedding of MAP in feces, internal condition, gross lesions and humoral immune responses up to 120 days (at each interval of 30 days). At the end of 120 days trial, there was marked overall improvement in physical condition and body weights of vaccinated goats as compared to 'Control' goats. Vaccinated goats gained significantly (P < 0.05) higher body weights, hardly exhibited any lesions characteristic of JD, had significantly higher (P < 0.01) antibody titers and shedding of MAP was significantly (P < 0.01) reduced. Few of the vaccinated goats were positive for MAP DNA in faecal PCR and blood PCR before vaccination. However, all were found as negative at 120 days post vaccination (DPV). Overall vaccine exhibited effective in restriction of MAP infection and significant improvement in production parameters and reduction in mortality and morbidity due to JD. The trial in the herd will be continued.



1447 Franco, M.M.J., Paes, A.C., Ribeiro, M.G., Pantoja, J.C.D., Santos, A.C.B., Miyata, M., Leite, C.Q.F., Motta, R.G., Listoni, F.J.P.

Occurrence of mycobacteria in bovine milk samples from both individual and collective bulk tanks at farms and informal markets in the southeast region of Sao Paulo, Brazil

Bmc Veterinary Research, (2013) 9, Article Number: 85 DOI: 10.1186/1746-6148-9-85
Published: APR 24 2013-Background: Mycobacterium spp. is one of the most important species of zoonotic pathogens that can be transmitted from cattle to humans. The presence of these opportunistic, pathogenic bacteria in bovine milk has emerged as a public-health concern, especially among individuals who consume raw milk and related dairy products. To address this concern, the Brazilian control and eradication program focusing on bovine tuberculosis, was established in 2001. However, bovine tuberculosis continues to afflict approximately 1,3 percent of the cattle in Brazil. In the present study, 300 samples of milk from bovine herds, obtained from both individual and collective bulk tanks and informal points of sale, were cultured on Lowenstein-Jensen and Stonebrink media. Polymerase chain reaction (PCR)-based tests and restriction-enzyme pattern analysis were then performed on the colonies exhibiting phenotypes suggestive of Mycobacterium spp., which were characterized as acid-fast bacilli. Results: Of the 300 bovine milk samples that were processed, 24 were positively identified as Mycobacterium spp. Molecular identification detected 15 unique mycobacterial species: Mycobacterium bovis, M. gordonae, M. fortuitum, M. intracellulare, M. flavescens, M. duvalii, M. haemophilum, M. immunogenum, M. lentiflavum, M. mucogenicum, M. novocastrense, M. parafortuitum, M. smegmatis, M. terrae and M. vaccae. The isolation of bacteria from the various locations occurred in the following proportions: 9 percent of the individual bulk-tank samples, 7 percent of the collective bulk-tank samples and 8 percent of the informal-trade samples. No statistically significant difference was observed between the presence of Mycobacterium spp. in the three types of samples collected, the milk production profiles, the presence of veterinary assistance and the reported concerns about bovine tuberculosis prevention in the herds. Conclusion: The microbiological cultures associated with PCR-based identification tests are possible tools for the investigation of the presence of Mycobacterium spp. in milk samples. Using these methods, we found that the Brazilian population may be regularly exposed to mycobacteria by consuming raw bovine milk and related dairy products. These evidences reinforces the need to optimize quality programs of dairy products, to intensify the sanitary inspection of these products and the necessity of further studies on the presence of Mycobacterium spp. in milk and milk-based products.

1448 O'Brien, R. , Hughes, A., Liggett, S., Griffin, F.

Composite testing for ante-mortem diagnosis of Johne's disease in farmed New Zealand deer: correlations between bacteriological culture, histopathology, serological reactivity and faecal shedding as determined by quantitative PCR

Bmc Veterinary Research, (2013) 9, Article Number: 72 DOI: 10.1186/1746-6148-9-72
Published: APR 10 2013-Background: In the absence of overt clinical signs of Johne's Disease (JD), laboratory based tests have largely been limited to organism detection via faecal culture or PCR and serological tests for antibody reactivity. In this study we describe the application of quantitative faecal PCR for the detection of Mycobacterium avium subsp. paratuberculosis (MAP) in New Zealand farmed deer to quantify the bacterial load in cervine faecal samples as an adjunct to an existing serodiagnostic test (Paralisa (TM)) tailored for JD diagnosis in deer. As ELISA has potential as a cheap, high throughput screening test for JD, an attempt was made to assess the sensitivity, specificity and positive/negative predictive (PPV/NPV) values of Paralisa (TM) for estimating levels of faecal shedding of MAP as a basis for JD management in deer. Results: Correlations were made between diagnostic tests (ELISA, qPCR, culture and histopathology) to establish the precision and predictive values of individual tests. The findings from this study suggest there is strong correlation between bacterial shedding, as determined by faecal qPCR, with both culture ($r = 0.9325$) and histopathological lesion severity scoring ($r = 0.7345$). Correlation between faecal shedding and ELISA reactivity in deer was weaker with values of $r = 0.4325$ and $r = 0.4006$ for Johnin and Protoplasmic antigens, respectively. At an ELISA Unit (EU) cutoff of >50 (Johnin antigen)



the PPV of Paralisa (TM) for significant faecal shedding in deer (>10(4) organisms/g) was moderate (0.55) while the NPV was higher (0.89). At an EU cutoff of ≥ 150 , the PPV for shedding >10(5) organisms/g rose to 0.88, with a corresponding NPV of 0.85. Conclusions: The evidence available from this study suggests that Paralisa (TM) used at a cutoff of 50EU could be used to screen deer herds for MAP infection with sequential qPCR testing used to cull all Paralisa (TM) positive animals that exhibit significant MAP faecal shedding.

1449 Stabel, J.R., Waters, W.R., Bannantine, J.P., Palmer, M.V.

Disparate Host Immunity to Mycobacterium avium subsp paratuberculosis Antigens in Calves Inoculated with M. avium subsp paratuberculosis, M. avium subsp avium, M. kansasii, and M. bovis

Clinical and Vaccine Immunology, (2013) 20, 848-857

The cross-reactivity of mycobacterial antigens in immune-based diagnostic assays has been a major concern and a criticism of the current tests that are used for the detection of paratuberculosis. In the present study, Mycobacterium avium subsp. paratuberculosis recombinant proteins were evaluated for antigenic specificity compared to a whole-cell sonicate preparation (MPS). Measures of cell-mediated immunity to M. avium subsp. paratuberculosis antigens were compared in calves inoculated with live M. avium subsp. paratuberculosis, M. avium subsp. avium (M. avium), Mycobacterium kansasii, or Mycobacterium bovis. Gamma interferon (IFN-gamma) responses to MPS were observed in all calves that were exposed to mycobacteria compared to control calves at 4 months postinfection. Pooled recombinant M. avium subsp. paratuberculosis proteins also elicited nonspecific IFN-gamma responses in inoculated calves, with the exception of calves infected with M. bovis. M. avium subsp. paratuberculosis proteins failed to elicit antigen-specific responses for the majority of immune measures; however, the expression of CD25 and CD26 was upregulated on CD4, CD8, gamma/delta (gamma delta) T, and B cells for the calves that were inoculated with either M. avium subsp. paratuberculosis or M. avium after antigen stimulation of the cells. Stimulation with MPS also resulted in the increased expression of CD26 on CD45RO(+) CD25(+) T cells from calves inoculated with M. avium subsp. paratuberculosis and M. avium. Although recombinant proteins failed to elicit specific responses for the calves inoculated with M. avium subsp. paratuberculosis, the differences in immune responses to M. avium subsp. paratuberculosis antigens were dependent upon mycobacterial exposure. The results demonstrated a close alignment in immune responses between calves inoculated with M. avium subsp. paratuberculosis and those inoculated with M. avium that were somewhat disparate from the responses in calves infected with M. bovis, suggesting that the biology of mycobacterial infection plays an important role in diagnosis.

1450 Santema, W. , Rutten, V., Segers, R., Poot, J., Hensen, S., Heesterbeek, H., Koets, A.

Postexposure Subunit Vaccination against Chronic Enteric Mycobacterial Infection in a Natural Host

Infection and Immunity, (2013) 81, 1990-1995

The control of chronic bacterial diseases with high prevalence in areas of endemicity would strongly benefit from availability of postexposure vaccines. The development of these vaccines against mycobacterial infections, such as (para)tuberculosis, is hampered by lack of experience in natural hosts. aratuberculosis in cattle is both a mycobacterial disease of worldwide importance and a natural host model for mycobacterial infections in general. The present study showed beneficial effects of therapeutic heat shock protein 70 (Hsp70) vaccination in cattle with naturally acquired chronic infection with Mycobacterium avium subsp. paratuberculosis. Vaccination-induced protection was associated with antibody responses, rather than with induction of specific T helper 1 cells. Targeted therapeutic postexposure vaccination complementary to selective use of antibiotics could be an effective approach for control of chronic mycobacterial infections.



1451 Ghosh, P., Wu, C.W., Talaat, A.M.

Key Role for the Alternative Sigma Factor, SigH, in the Intracellular Life of Mycobacterium avium subsp paratuberculosis during Macrophage Stress

Infection and Immunity, (2013) 81, 2242-2257

Mycobacterium avium subsp. paratuberculosis causes Johne's disease, an enteric infection in cattle and other ruminants, greatly afflicting the dairy industry worldwide. Once inside the cell, M. avium subsp. paratuberculosis is known to survive harsh microenvironments, especially those inside activated macrophages. To improve our understanding of M. avium subsp. paratuberculosis pathogenesis, we examined phagosome maturation associated with transcriptional responses of M. avium subsp. paratuberculosis during macrophage infection. Monitoring cellular markers, only live M. avium subsp. paratuberculosis bacilli were able to prevent phagosome maturation and reduce its acidification. On the transcriptional level, over 300 M. avium subsp. paratuberculosis genes were significantly and differentially regulated in both naive and IFN-gamma-activated macrophages. These genes include the sigma factor H (sigH) that was shown to be important for M. avium subsp. paratuberculosis survival inside gamma interferon (IFN-gamma)-activated bovine macrophages. Interestingly, a sigH-knockout mutant showed increased sensitivity to a sustained level of thiol-specific oxidative stress. Large-scale RNA sequence analysis revealed that a large number of genes belong to the sigH regulon, especially following diamide stress. Genes involved in oxidative stress and virulence were among the induced genes in the sigH regulon with a putative consensus sequence for SigH binding that was recognized in a subset of these genes (n = 30), suggesting direct regulation by SigH. Finally, mice infections showed a significant attenuation of the Delta sigH mutant compared to its parental strain, suggesting a role for sigH in M. avium subsp. paratuberculosis virulence. Such analysis could identify potential targets for further testing as vaccine candidates against Johne's disease.

1452 Johnston, C., Douarre, P.E., Soulimane, T., Pletzer, D., Weingart, H., MacSharry, J., Coffey, A., Sleator, R.D., O'Mahony, J.

Codon optimisation to improve expression of a Mycobacterium avium ssp paratuberculosis-specific membrane-associated antigen by Lactobacillus salivarius

Pathogens and Disease, (2013) 68, 27-38

Subunit and DNA-based vaccines against Mycobacterium avium ssp. paratuberculosis (MAP) attempt to overcome inherent issues associated with whole-cell formulations. However, these vaccines can be hampered by poor expression of recombinant antigens from a number of disparate hosts. The high G+C content of MAP invariably leads to a codon bias throughout gene expression. To investigate if the codon bias affects recombinant MAP antigen expression, the open reading frame of a MAP-specific antigen MptD (MAP3733c) was codon optimised for expression against a Lactobacillus salivarius host. Of the total 209 codons which constitute MAP3733c, 172 were modified resulting in a reduced G+C content from 61% for the native gene to 32.7% for the modified form. Both genes were placed under the transcriptional control of the PnisA promoter; allowing controlled heterologous expression in L.salivarius. Expression was monitored using fluorescence microscopy and microplate fluorometry via GFP tags translationally fused to the C-termini of the two MptD genes. A >37-fold increase in expression was observed for the codon-optimised MAP3733synth variant over the native gene. Due to the low cost and improved expression achieved, codon optimisation significantly improves the potential of L.salivarius as an oral vaccine stratagem against Johne's disease.

1453 Miller, R.S., Farnsworth, M.L., Malmberg, J.L.

Diseases at the livestock-wildlife interface: Status, challenges, and opportunities in the United States

Preventive Veterinary Medicine, (2013) 110, 119-132

In the last half century, significant attention has been given to animal diseases; however, our understanding of disease processes and how to manage them at the livestock-wildlife interface remains limited. In this study, we conduct a systematic review of the scientific literature to evaluate the status of diseases at the livestock-wildlife interface in the United States. Specifically, the goals of the literature review were three fold: first to evaluate domestic animal diseases currently found in the United States where wildlife may play a role; second to



identify critical issues faced in managing these diseases at the livestock-wildlife interface; and third to identify potential technical and policy strategies for addressing these issues. We found that of the 86 avian, ruminant, swine, poultry, and lagomorph diseases that are reportable to the World Organization for Animal Health (OIE), 53 are present in the United States; 42 (79%) of these have a putative wildlife component associated with the transmission, maintenance, or life cycle of the pathogen; and 21 (40%) are known to be zoonotic. At least six of these reportable diseases bovine tuberculosis, paratuberculosis, brucellosis, avian influenza, rabies, and cattle fever tick (vector control) have a wildlife reservoir that is a recognized impediment to eradication in domestic populations. The complex nature of these systems highlights the need to understand the role of wildlife in the epidemiology, transmission, and maintenance of infectious diseases of livestock. Successful management or eradication of these diseases will require the development of cross-discipline and institutional collaborations. Despite social and policy challenges, there remain opportunities to develop new collaborations and new technologies to mitigate the risks posed at the livestock-wildlife interface. Published by Elsevier B.V.

1454 Okuni, J.B. , Reinacher, M., Loukopoulos, P., Ojok, L.

Prevalence and spectrum of Johne's disease lesions in cattle slaughtered at two abattoirs in Kampala, Uganda

Tropical Animal Health and Production, (2013) 45, 1197-1202

This study was conducted to determine the prevalence and characteristics of Johne's disease (JD) lesions in Ugandan cattle slaughtered at two of the main abattoirs in Kampala. Ileocaecal junction and the associated lymph nodes of 1,022 cattle were examined for gross and microscopic lesions, followed by Ziehl Neelsen staining of the tissues bearing lesions. Confirmation of *Mycobacterium avium* subsp. paratuberculosis infection was done in some of the tissues using culture and IS900 PCR. The lesions were then described, characterised and tabulated. Characteristic Johne's disease granulomas were found in 4.7% of the samples examined, derived from Zebu, Ankole longhorn, Friesian breeds of cattle and their crosses. Lesions were found both in the lymph nodes and ileocaecal junction mucosa. The lesions tended to be more severe in the lymph node than in the mucosa. There were also some unique and atypical lesions found in association with Johne's disease granulomas. The diagnostic values of various gross lesions and criteria of lesion classifications and diagnosis are revisited and discussed based on the findings of this study. The prevalence of Johne's disease lesions among slaughtered cattle in Kampala's two abattoirs indicates that the disease is well established in the cattle population in the country. The diverse manifestations in lesions of JD need to be considered when making histological diagnosis in tissues where the disease is suspected.

1455 Khol, J.L., Wassertheurer, M., Sodoma, E., Revilla-Fernandez, S., Damoser, J., Oserreicher, E., Dunser, M., Kleb, U., Baumgartner, W.

Long-term detection of *Mycobacterium avium* subspecies paratuberculosis in individual and bulk tank milk from a dairy herd with a low prevalence of Johne's disease

Journal of Dairy Science, (2013) 96, 3517-3524

Mycobacterium avium ssp. paratuberculosis (MAP) causes Johne's disease (JD) in ruminants and is shed into the milk of infected cows, which contributes to the controversial discussion about a possible link between MAP and Crohn's disease in humans. The aim of the study was to investigate the risk for the entry of MAP in the food chain via milk from dairy farms with subclinical JD. Therefore, the occurrence of MAP in the milk of a dairy herd with a low prevalence of JD was studied in single and bulk tank milk samples over a period of 23 mo and compared with MAP shedding into feces. Milk, fecal, and blood samples were taken from all cows older than 1.5 yr of age at the beginning and the end of the trial and analyzed for MAP or specific antibodies. In addition, 63 cows (33 MAP infected and 30 MAP noninfected) were selected for monthly sampling. Raw and pasteurized bulk tank milk samples were collected on a monthly basis. The milk samples were tested for MAP by real-time quantitative PCR (qPCR), and the fecal samples were tested for bacterial shedding by qPCR or solid culture.



Based on the results of the herd investigations, the prevalence of cows shedding MAP was around 5%; no cases of clinical JD were observed during the study period. The results of the ELISA showed high variation, with 2.1 to 5.1% positive milk samples and 14.9 to 18.8% ELISA-positive blood samples. Monthly milk sampling revealed low levels of MAP shedding into the individual milk samples of both MAP-infected and noninfected cows, with only 13 cows shedding the bacterium into milk during the study period. *Mycobacterium avium* ssp. paratuberculosis was not detected by qPCR in any raw or pasteurized bulk tank milk sample throughout the study. A significant positive association could be found between MAP shedding into milk and feces. From the results of the present study, it can be concluded that MAP is only shed via milk in a small proportion of cows with subclinical JD for a limited period of time and is diluted below the detection level of qPCR within the bulk tank milk of these herds. These findings indicate that dairy herds subclinically infected with JD pose only a minor source for human MAP consumption with milk and milk products.

CGNI SUBSCRIPTION IS FREE. To change your e-mail address, to change the selected fields of interest, or to unsubscribe visit [REGISTRATION](#).

Contributions, comments and requests of the subscribers are welcome. Ask [the Editor](#) to publish your query or contribution.

See more information on [NETWORK WEBPAGE](#).