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2013-03-25-012 Paratuberculosis databases updated (2013-03-20)
To: (04) Mycobacterial diseases; (12) Scientific Information, research and education; .

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New publications in the PARATUBERCULOSIS database (1375-1386)

1375   Charavaryamath, C., Gonzalez-Cano, P., Fries, P., Gomis, S., Doig, K., Scruten, E., Potter, A., Napper, S., Griebel, P.J.

Host Responses to Persistent Mycobacterium avium Subspecies paratuberculosis Infection in Surgically Isolated Bovine Ileal Segments
Clinical and Vaccine Immunology, (2013)   20, 156-165

A lack of appropriate disease models has limited our understanding of the pathogenesis of persistent enteric infections with Mycobacterium avium subsp. paratuberculosis. A model was developed for the controlled delivery of a defined dose of M. avium subsp. paratuberculosis to surgically isolated ileal segments in newborn calves. The stable intestinal segments enabled the characterization of host responses to persistent M. avium subsp. paratuberculosis infections after a 9-month period, including an analysis of local mucosal immune responses relative to an adjacent uninfected intestinal compartment. M. avium subsp. paratuberculosis remained localized at the initial site of intestinal infection and was not detected by PCR in the mesenteric lymph node. M. avium subsp. paratuberculosis-specific T cell proliferative responses included both CD4 and gamma delta T cell receptor (gamma delta TcR) T cell responses in the draining mesenteric lymph node. The levels of CD8(+) and gamma delta TcR+ T cells increased significantly (P<0.05) in the lamina propria, and M. avium subsp. paratuberculosis-specific tumor necrosis factor alpha (TNF-alpha) and gamma interferon secretion by lamina propria leukocytes was also significantly (P<0.05) increased. There was a significant (P<0.05) accumulation of macrophages and dendritic cells (DCs) in the lamina propria, but the expression of mucosal toll-like receptors 1 through 10 was not significantly changed by M. avium subsp. paratuberculosis infection. In conclusion, surgically isolated ileal segments provided a model system for the establishment of a persistent and localized enteric M. avium subsp. paratuberculosis infection in cattle and facilitated the analysis of M. avium subsp. paratuberculosis-specific changes in mucosal leukocyte phenotype and function. The accumulation of DC subpopulations in the lamina propria suggests that further investigation of mucosal DCs may provide insight into host responses to M. avium subsp. paratuberculosis infection and improve vaccine strategies to prevent M. avium subsp. paratuberculosis infection/

1376   Iakhiaeva, E., McNulty, S., Elliott, B.A.B., Falkinham, J.O., Williams, M.D., Vasireddy, R., Wilson, R.W. , Turenne, C., Wallace, R.J.

Mycobacterial Interspersed Repetitive-Unit-Variable-Number Tandem-Repeat (MIRU-VNTR) Genotyping of Mycobacterium intracellulare for Strain Comparison with Establishment of a PCR-Based Database
Journal of Clinical Microbiology, (2013) 51, 409-416

Strain comparison is important to population genetics and to evaluate relapses in patients with Mycobacterium avium complex (MAC) lung disease, but the "gold standard" of pulsed-field gel electrophoresis (PFGE) is time-consuming and complex. We used variable-number tandem repeats (VNTR) for fingerprinting of respiratory isolates of M. intracellulare from patients with underlying bronchiectasis, to establish a nonsequence-based database for population analysis. Different genotypes identified by PFGE underwent species identification using a 16S rRNA gene multiplex PCR. Genotypes of M. intracellulare were confirmed by internal transcribed spacer 1 (ITS1) sequencing and characterized using seven VNTR primers. The pattern of VNTR amplicon sizes and repeat number defined each specific VNTR type. Forty-two VNTR types were identified among 84 genotypes. PFGE revealed most isolates with the same VNTR type to be clonal or exhibit similar grouping of bands. Repetitive seque nce-based PCR (rep-PCR) showed minimal pattern diversity between VNTR types compared to PFGE.

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Fingerprinting of relapse isolates from 31 treated patients using VNTR combined with 16S multiplex PCR unambiguously and reliably distinguished different genotypes from the same patient, with results comparable to those of PFGE. VNTR for strain comparison is easier and faster than PFGE, is as accurate as PFGE, and does not require sequencing. Starting with a collection of 167 M. intracellulare isolates, VNTR distinguished M. intracellulare into 42 clonal groups. Comparison of isolates from different geographic areas, habitats, and clinical settings is now possible/

1377   Lu, Z., Schukken, Y.H., Smith, R.L., Mitchell, R.M., Grohn, Y.T.

Impact of imperfect Mycobacterium avium subsp paratuberculosis vaccines in dairy herds: A mathematical modeling approach
Preventive Veterinary Medicine, (2013) 108, 148-158

The objective of this study was to investigate the potential impacts of imperfect Mycobacterium avium subsp. paratuberculosis (MAP) vaccines on the dynamics of MAP infection in US dairy herds using a mathematical modeling approach. Vaccine-based control programs have been implemented to reduce the prevalence of MAP infection in some dairy herds; however, MAP vaccines are imperfect. Vaccines can provide partial protection for susceptible calves, reduce the infectiousness of animals shedding MAP, lengthen the latent period of infected animals, slow the progression from low shedding to high shedding in infectious animals, and reduce clinical disease. To quantitatively study the impacts of imperfect MAP vaccines, we developed a deterministic multi-group vaccination model and performed global sensitivity analyses. Our results explain why MAP vaccination might have a beneficial, negligible, or detrimental effect in the reduction of prevalence and show that vaccines that are bene ficial to individual animals may not be useful for a herd-level control plan. The study suggests that high efficacy vaccines that are aimed at reducing the susceptibility of the host are the most effective in controlling MAP transmission. This work indicates that MAP vaccination should be integrated into a comprehensive control program that includes test-and-cull intervention and improved calf rearing management. (c) 2012 Elsevier B.V. All rights reserved/

1378   Lombard, J.E., Gardner, I.A., Jafarzadeh, S.R., Fossler, C.P., Harris, B., Capsel, R.T., Wagner, B.A., Johnson, W.O.

Herd-level prevalence of Mycobacterium avium subsp paratuberculosis infection in United States dairy herds in 2007
Preventive Veterinary Medicine, (2013) 108, 234-238

Testing of composite fecal (environmental) samples from high traffic areas in dairy herds has been shown to be a cost-effective and sensitive method for classification of herd status for Mycobacterium avium subsp. paratuberculosis (MAP). In the National Animal Health Monitoring System's (NAHMS) Dairy 2007 study, the apparent herd-level prevalence of MAP was 70.4% (369/524 had >= 1 culture-positive composite fecal samples out of 6 tested). Based on these data, the true herd-level prevalence (HP) of MAP infection was estimated using Bayesian methods adjusting for the herd sensitivity (HSe) and herd specificity (HSp) of the test method. The Bayesian prior for HSe of composite fecal cultures was based on data from the NAHMS Dairy 2002 study and the prior for HSp was based on expert opinion. The posterior median HP (base model) was 91.1% (95% probability interval, 81.6 to 99.3%) and estimates were most sensitive to the prior for HSe. The HP was higher than estimated from the NAHMS Dairy 1996 and 2002 studies but estimates are not directly comparable with those of prior NAHMS studies because of the different testing methods and criteria used for herd classification. Published by Elsevier B.V/

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1379 Salem, M., Heydel, C., El-Sayed, A., Ahmed, S.A., Zschock, M., Baljer, G.
Mycobacterium avium subspecies paratuberculosis: an insidious problem for the ruminant industry
Tropical Animal Health and Production, (2013) 45, 351-366

Mycobacterium avium subspecies paratuberculosis is considered as one of the most serious problems affecting the world's ruminant industry due to its significant impact on the global economy and the controversial issue that it may be pathogenic for humans. M. avium subspecies paratuberculosis is the causative agent of Johne's disease in animals and might be implicated in cases of human Crohn's disease. We provide an insight into M. avium subspecies paratuberculosis from some bacteriological, clinical, and molecular epidemiological perspectives/

1380 Aly, S.S., Anderson, R.J., Whitlock, R.H., Fyock, T.L., McAdams, S.C., Byrem, T.M., Jiang, J.M., Adaska, J.M., Gardner, I.A.

Cost-effectiveness of diagnostic strategies to identify Mycobacterium avium subspecies paratuberculosis super-shedder cows in a large dairy herd using antibody enzyme-linked immunosorbent assays, quantitative real-time polymerase chain reaction, and bacterial culture
Journal of Veterinary Diagnostic Investigation, (2012) 24, 821-832

Diagnostic strategies to detect Mycobacterium avium subsp. paratuberculosis (MAP) super-shedder cows in dairy herds have been minimally studied. The objective of the current study was to compare the cost-effectiveness of strategies for identification of MAP super-shedders on a California dairy herd of 3,577 cows housed in free-stall pens. Eleven strategies that included serum or milk enzyme-linked immunosorbent assay (ELISA), quantitative real-time polymerase chain reaction (qPCR) or culture of environmental samples, pooled or individual cow fecal samples, or combinations thereof were compared. Nineteen super-shedders (0.5%) were identified by qPCR and confirmed by culture as cows shedding >= 10,000 colony forming units (CFU)/g feces (median of 30,000 CFU/g feces). A stratified random sample of the study herd based on qPCR results of fecal pools was the most sensitive (74%) strategy and had the highest cost ($5,398/super-shedder). The reference strategy with the lowest cost ($1,230/super-shedder) and sensitivity (47%) included qPCR testing of fecal samples from ELISA-positive lactating (milk) and nonlactating (serum) cows housed in pens with the highest MAP bioburden. The most cost-effective alternative to the reference was to perform qPCR testing of fecal samples from ELISA-positive cows (milk and serum for milking and dry cows, respectively) for a sensitivity of 68% and cost of $2,226/super-shedder. In conclusion, diagnostic strategies varied in their cost-effectiveness depending on the tests, specimen type, and labor costs. Initial qPCR testing of environmental samples from free-stall pens to target cows in pens with the highest MAP bioburden for further testing can improve the cost-effectiveness of strategies for super-shedder identification/

1381 Pruvot, M., Forde, T.L., Steele, J., Kutz, S.J., De Buck, J., van der Meer, F., Orsel, K.

The modification and evaluation of an ELISA test for the surveillance of Mycobacterium avium subsp paratuberculosis infection in wild ruminants
Bmc Veterinary Research, (2013) 9, Article Number: 5 DOI: 10.1186/1746-6148-9-5
Published: JAN 9 2013-

Background: Enzyme-linked immunosorbent assay (ELISA) is often used to test wildlife samples for Mycobacterium avium subsp. paratuberculosis (MAP) infection. However, commercially available kits are only validated for use with domestic ruminant species. A literature review was performed to document the current use of MAP serum ELISA in wild and semi-domestic ruminants. We then modified and evaluated a commercial ELISA kit (IDEXX Mycobacterium paratuberculosis Antibody Test Kit) for use with species for which it was not originally developed: elk (Cervus elaphus), bison (Bison bison) and caribou (Rangifer tarandus). We tested the affinity of different conjugates for immunoglobulin G (IgG) isolated

3

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from these species, performed checkerboard tests to determine the optimal dilutions of samples and conjugates, and established cut-off values using two different methods: a Receiver Operational Curve on a panel of known samples for elk, and an alternate method involving a panel of unknown serum samples for the three species. Results: We found that the anti-bovine conjugate included in the IDEXX ELISA kit has limited affinity for elk, bison, and caribou IgG. Protein G showed good affinity for IgG of all three species, while anti-deer conjugate also bound elk and caribou IgG. Using Protein G with elk serum, a cut-off sample-to-positive (S/ P) value of 0.22 was selected, resulting in a sensitivity and specificity of 73% and 90%, respectively, whereas, using an anti-deer conjugate with elk serum, an S/ P cut-off value of 0.29 gave a sensitivity of 68%, with 100% specificity. Cut-off values for bison and caribou using the Protein G conjugate were 0.17 and 0.25 respectively. Conclusions: Due to incomplete reporting and a lack of test validation, it is difficult to critically appraise results of many sero-surveys that have previously been done for MAP in wildlife. Commercial ELISA kits may have limited or no capacity to detect antibodies from species other t han for which they were developed. In order to generate reliable test results, it is essential to evaluate the test and perform modifications if deemed necessary. Despite the challenges inherent to wildlife diagnostics, we have shown that several methods can be used to improve confidence in test results/

1382   Yildirim, D., Civelek, T.

Prevalence of Subclinical Paratuberculosis in Dairy Cattle in Usak Region
Kafkas Universitesi Veteriner Fakultesi Dergisi, (2013) 19, 121-126

Paratuberculosis caused by Mycobacterium avium subs. paratuberculosis a chronic, inflammatory and fatal disease of ruminants. The infection is characterized by chronic "subclinic" phase. Cattle in this phase is capable of infecting other animals in the herd. Although the presence of paratuberculosis has been known, the scientific studies on the disease and the prevalence appears to be scarced in Turkey. Thus, there is no scientific report on prevalence of the disease in dairy cattle in Usak region. In this study, it was aimed to determine the prevalence of subclinic paratuberculosis in dairy cattle farming in Usak region in Turkey. In the study a total of 200 Holstein dairy cattle aged between 3-7 years clinically healthy with optimum milk yield and body condition score were used. The MAP were identified in feces and milk samples using direct bacterioscopy technique, and in positive samples by culturing and Polymerase Chain Reaction (outer and nested) techniques. In Usak region in dairy cattle the prevalence of paratuberculosis was determined to be 17% by Ziehl-Neelsen staining, 9.5% by Outer Polymerase Chain Reaction and 20% Nested Polymerase Chain Reaction in feces; 4% according to bacteriologic culture results; 15.5% by Ziehl-Neelsen staining, 5.5% by Outer Polymerase Chain Reaction and 17.5% by Nested Polymerase Chain Reaction in milk samples and 2.5% according to bacteriologic culture results/

1383   Davidson, R.S., McKendrick, I.J., Wood, J.C., Marion, G., Greig, A., Stevenson, K., Sharp, M., Hutchings, M.R.

Accounting for uncertainty in model-based prevalence estimation: paratuberculosis control in dairy herds
Bmc Veterinary Research, (2012) 8, Article Number: 159   DOI: 10.1186/1746-6148-8-159
Published: SEP 10 2012-

Background: A common approach to the application of epidemiological models is to determine a single (point estimate) parameterisation using the information available in the literature. However, in many cases there is considerable uncertainty about parameter values, reflecting both the incomplete nature of current knowledge and natural variation, for example between farms. Furthermore model outcomes may be highly sensitive to different parameter values. Paratuberculosis is an infection for which many of the key parameter values are poorly understood and highly variable, and for such infections there is a need to develop and apply statistical techniques which make maximal use of available data. Results: A technique based on Latin hypercube sampling combined with a novel reweighting method was developed which

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enables parameter uncertainty and variability to be incorporated into a model-based framework for estimation of prevalence. The method was evaluated by applying it to a simulation of paratuberculosis in dairy herds which combines a continuous time stochastic algorithm with model features such as within herd variability in disease development and shedding, which have not been previously explored in paratuberculosis models. Generated sample parameter combinations were assigned a weight, determined by quantifying the model's resultant ability to reproduce prevalence data. Once these weights are generated the model can be used to evaluate other scenarios such as control options. To illustrate the utility of this approach these reweighted model outputs were used to compare standard test and cull control strategies both individually and in combination with simple husbandry practices that aim to reduce infection rates. Conclusions: The technique developed has been shown to be applicable to a complex model incorporating realistic control options. For models where parameters are not well known or subject to significant variability, the reweighti ng scheme allowed estimated distributions of parameter values to be combined with additional sources of information, such as that available from prevalence distributions, resulting in outputs which implicitly handle variation and uncertainty. This methodology allows for more robust predictions from modelling approaches by allowing for parameter uncertainty and combining different sources of information, and is thus expected to be useful in application to a large number of disease systems/

1384 Sorge, U.S. , Molitor, T., Linn, J., Gallaher, D., Wells, S.W.
Cow-level association between serum 25-hydroxyvitamin D concentration and Mycobacterium avium subspecies paratuberculosis antibody seropositivity: A pilot study
Journal of Dairy Science, (2013) 96, 1030-1037

Vitamin D deficiency has been associated with various human diseases. Therefore, the objective of this study was to evaluate the cow-level association between serum 25-hydroxyvitamin D [25(OH)D] concentration and Mycobacterium avium ssp. paratuberculosis (MAP) seropositivity of dairy cows, adjusting for diet, breed, hair coat color, stage of lactation, reproductive status, and cow age. The sera of 80 MAP antibody ELISA-positive and 80 test-negative herd mates from 5 Minnesota dairy herds were analyzed for 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)(2)D]. The cows' age, production records, and hair coat color were recorded. Additionally, feed samples were obtained and analyzed for vitamin D-2 and vitamin D-3 content. A linear mixed model was used to identify potential predictors for serum 25(OH)D concentration, accounting for herd of origin. The majority of rations analyzed had over 22,000 IU of vitamin D/day (maximum: 52,000 IU/d) and the study cows' average ser um 25(OH)D concentration was 62.5 +/- 13.8 ng/mL. Serum ELISA-positive cows had, on average, 5.3 ng/mL lower 25(OH)D serum levels than test-negative herd mates. The reproductive status of cows was also associated with the 25(OH)D levels, with fresh cows having the lowest serum concentration. In this cross-sectional study, a temporal or causal association between MAP antibody ELISA status and serum 25(OH)D concentration could not be evaluated. In addition, the high levels of vitamin D in the rations of participating farms and the average 25(OH)D serum concentration suggest that additional supplementation with vitamin D in the ration is likely to be ineffective/

1385 Biet, F., Sevilla, I.A., Cochard, T., Lefrancois, L.H., Garrido, J.M., Heron, I., Juste, R.A., McLuckie, J., Thibault, V.C., Supply, P., Collins, D.M., Behr, M.A., Stevenson, K.

Inter- and Intra-subtype genotypic differences that differentiate Mycobacterium avium subspecies paratuberculosis strains
Bmc Microbiology, (2012) 12, Article Number: 264   DOI: 10.1186/1471-2180-12-264
Published: NOV 19 2012---

Background: Mycobacterium avium subspecies paratuberculosis (Map) is the aetiological agent of Johne's disease or paratuberculosis and is included within the Mycobacterium avium complex (MAC). Map strains are of two major types often referred to as 'Sheep' or 'S-type' and 'Cattle' or 'C-type'. With the advent of more discriminatory typing techniques it has been

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possible to further classify the S-type strains into two groups referred to as Type I and Type III. This study was undertaken to genotype a large panel of S-type small ruminant isolates from different hosts and geographical origins and to compare them with a large panel of well documented C-type isolates to assess the genetic diversity of these strain types. Methods used included Mycobacterial Interspersed Repetitive Units - Variable-Number Tandem Repeat analysis (MIRU-VNTR), analysis of Large Sequence Polymorphisms by PCR (LSP analysis), Single Nucleotide Polymorphism (SNP) analy sis of gyr genes, Pulsed-Field Gel Electrophoresis (PFGE) and Restriction Fragment Length Polymorphism analysis coupled with hybridization to IS900 (IS900-RFLP) analysis. Results: The presence of LSP(A)4 and absence of LSP(A)20 was confirmed in all 24

Map S-type strains analysed. SNPs within the gyr genes divided the S-type strains into types I and III. Twenty four PFGE multiplex profiles and eleven different IS900-RFLP profiles were identified among the S-type isolates, some of them not previously published. Both PFGE and IS900-RFLP segregated the S-type strains into types I and III and the results concurred with those of the gyr SNP analysis. Nine MIRU-VNTR genotypes were identified in these isolates. MIRU-VNTR analysis differentiated Map strains from other members of Mycobacterium avium Complex, and Map S-type from C-type but not type I from III. Pigmented Map isolates were found of type I or III. Conclusion: This is the largest panel of S-type strains investigated to dat e. The S-type strains could be further divided into two subtypes, I and III by some of the typing techniques (IS900-RFLP, PFGE and SNP analysis of the gyr genes). MIRU-VNTR did not divide the strains into the subtypes I and III but did detect genetic differences between isolates within each of the subtypes. Pigmentation is not exclusively associated with type I strains/

1386   Stabel, J.R., Barnhill, A., Bannantine, J.P., Chang, Y.F., Osman, M.A.

Evaluation of protection in a mouse model after vaccination with Mycobacterium avium subsp paratuberculois protein cocktails
Vaccine, (2012) 31, 127-134

Whole-cell vaccines successfully reduce signs of clinical disease and fecal shedding of Mycobacterium avium subsp. paratuberculosis (MAP), however, these vaccines have some limitations. The present study was conducted to identify MAP proteins that might be candidates for the development of an improved vaccine. MAP proteins were screened for immunogenicity in naturally infected cattle and selected based upon reactivity in the interferon-gamma (IFN-gamma) and Western blot assays. Proteins (MAP1087, MAP1204, MAP1272c, and MAP2077c) were arrayed into 4 overlapping cocktails containing 3 proteins each. The efficacy of the proteins within these cocktails as vaccine candidates was evaluated by subcutaneous immunization of mice, followed by challenge with live, virulent MAP. All MAP protein cocktails significantly reduced the recovery of live MAP from the ileum, while cocktails 1 and 3 reduced colonization in the liver. No significant differences were seen in the mesenteric lymph node or spleen, however, cocktail 1 reduced viable MAP in the mesenteric lymph node compared to other treatments. Stimulation of splenocytes upregulated antigen-specific IFN-gamma and IL-23 secretion in all treatment groups, regardless of vaccination. Interestingly. IL-4 was moderately downregulated for vaccinates compared to control infected mice. An increase in total CD25 expression was noted for 3 of the 4 vaccinate groups upon stimulation of splenocytes with a whole cell sonicate of MAP, with this effect becoming more significant within CD4CD25+ and CD8CD25+ subpopulations. The present study demonstrated that MAP proteins are useful as vaccine candidates to reduce MAP tissue burden. Published by Elsevier Ltd/

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