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New publications in the PARATUBERCULOSIS database (1080-1085)

Paratuberculosis in European wild rabbits from the Iberian Peninsula
Research in Veterinary Science, 91, 212-218

Of the non-ruminant wildlife species known to harbor Mycobacterium avium paratuberculosis (MAP), the rabbit (Oryctolagus cuniculus) is thought to pose the greatest risk of transmission to cattle. We analyzed 80 hunter-harvested wild rabbits from a core study area in southern Spain, and sera from 157 wild rabbits sampled opportunistically on seven additional sites. Gross lesions compatible with paratuberculosis were observed in two of 80 necropsied rabbits. Histopathology revealed focal to diffuse multibacillary MAP-compatible lesions in 8 of 10 rabbits examined. Presence of MAP was confirmed in one rabbit with gross lesions by positive amplification curves for both 15900 and ISMAP02. However, no isolate was obtained from 47 samples by culture. We adapted an indirect ELISA for the detection of MAP antibodies. At the established cut-off of 0.5, 6 of 237 wild rabbit sera (2.5%) yielded a positive ELISA result. Antibodies were detected in rabbits from 3 of 8 sampling sites. Considering the increasing relevance of MAP infection for animal health, these results open a challenging field for future research. (C) 2011 Elsevier Ltd. All rights reserved

High-resolution melting for analysis of short sequence repeats in Mycobacterium avium subsp paratuberculosis
Fems Microbiology Letters, 323, 151-154

Analysis of micro- and minisatellite loci is widely used in sub-typing of Mycobacterium avium subsp. paratuberculosis. Microsatellite (short sequence repeat, SSR) loci have shown highest discriminatory power, but direct sequencing of amplicons is required for correct assignment of the repeat number. We developed an alternative method to sequencing, focusing on the SSR8 locus (constituted by GGT triplets from three to six repeats). The approach is based on asymmetric quantitative PCR, followed by high-resolution melting analysis with unlabelled probes (UP-HRM). Data showed perfect concordance between direct sequencing and UP-HRM, which is faster, simpler and more cost effective

Effectiveness of combination of Mini-and Microsatellite loci to sub-type Mycobacterium avium subsp paratuberculosis Italian type C isolates
Bmc Veterinary Research, 7, Background: Mycobacterium avium subsp. paratuberculosis (Map) is the etiological agent of paratuberculosis. The aim of our study was to combine Mini- and Microsatellite loci analysis in order to explore the effectiveness of this sub-typing method in a group of Map isolates. For this purpose, 84 Italian Type C Map isolates, each from a different cattle herd, were submitted to MIRU-Variable-Number Tandem-Repeat (VNTRs) typing and Short Sequence repeats (SSRs) sequencing. Moreover, the method was used to analyse the variability inside 10 herds (from three to 50 isolates per herd). Results: The molecular sub-typing, carried out using three SSR and 10 MIRU-VNTR loci, differentiated the 84 isolates into 33 clusters, reaching a Simpson’s Discriminatory Index (SID) value of 0.952 (0.933 to 0.972, 95% confidence intervals). Among all considered loci, six (SSR2, MIRU2, SSR1, SSR8, VNTR3527 and VNTR1067) showed relevant allelic variability. Thirty-eight% of the isolates were clustered into four genotypes, differing from each other for the SSR2 locus. The other isolates, characterised by differences in two or more loci, were spread among the rest of the clusters. The intra-herd analysis revealed more than one genotype in most herds
with a similar distribution of clusters. Conclusions: Our results revealed the advantage of using both Mini- and Microsatellite approaches for successfully discriminating among Map Type C isolates from the same geographic area, host species and herd. These data suggest that the combination of loci here proposed could be a useful molecular tool for regional epidemiological studies.


Paratuberculosis (JD) is a chronic contagious inflammation of the intestines of ruminants that causes considerable economic losses. The diagnosis of MAP infections could be the basis for the development of a JD eradication programme. The aim of the study was to determine the seroprevalence of JD in cattle herds in two districts of north-eastern Poland. Serum samples taken from 1,203 cattle herds were subjected to a commercial ELISA test (MASA paratuberculosis screening test (R), Institut Pourquier). Animals with positive reactions to paratuberculosis were detected in 76 herds. Considerable differences between the two districts were observed in the results obtained: in District I the seroprevalence of JD amounted to 3.2%, whereas in District II it was 0.8%. The differences in seroprevalence were related to the size of the herds.


Stochastic simulation models are widely accepted as a means of assessing the impact of changes in daily management and the control of different diseases, such as paratuberculosis, in dairy herds. This paper summarises and discusses the assumptions of four stochastic simulation models and their use in the design of certification, surveillance, and control strategies for paratuberculosis in cattle herds. A detailed comparison is made between the Dutch JohneSSim and the Danish PTB-Simherd, using the same context of a set of control strategies in a typical Dutch/Danish herd. The conclusion is that while the models are somewhat different in their underlying principles and do put slightly different values on the different strategies, their overall findings are similar. Therefore, simulation models may be useful in planning paratuberculosis strategies in dairy herds, although as with all models caution is warranted when interpreting and generalising the results.


Macrophage migration inhibitory factor (MIF) is a unique pro-inflammatory cytokine whose chief functions include modulating TLR4 expression, and suppressing the anti-inflammatory effects of glucocorticoids. Not surprisingly, MIF is involved in a number of inflammatory diseases and single nucleotide polymorphisms (SNPs) have been implicated in modulating disease severity. The objective of the present study was to determine if SNPs in 50 region of bovine MIF affects its promoter activity. Three SNPs were identified, -1078A > G, -395A > G, and -400G > C, all of which fall within predicted transcription factor binding regions. Reporter gene assays indicate that the identified SNPs have a significant effect of modulating MIF promoter activity. Finally, gene association analysis suggests a significant relationship of -395A > G with the susceptibility to Mycobacterium avium ssp. paratuberculosis infection, the causative agent of Johne's disease. Given the relationships revealed in the current study, it is clear that the role of MIF in bovine diseases such as Johne's disease merits further investigation.

New publications in the CROHN’S DISEASE AND PARATUBERCULOSIS database (604-605)

Background: The prevalence of Crohn's disease (CD) is increased in patients with cystic fibrosis (CF). Anti-Saccharomyces cerevisiae antibodies (ASCA) have been suggested as a screening tool to detect CD in CF. Recently, several new anti-glycan antibodies have been reported in CD. Materials and methods: The sera of 119 CF patients of various age groups were prospectively screened for ASCA type IgG (gASCA), anti-laminaribioside carbohydrate IgG antibodies (ALCA), anti-chitobioside carbohydrate IgA antibodies (ACCA), and anti-mannobioside carbohydrate IgG antibodies (AMCA). The frequency of these anti-glycan antibodies was then compared in patients with CD, ulcerative colitis, rheumatoid arthritis and healthy volunteers. Results: A significant number of CF patients were positive for gASCA (51.3% [41.6-60.6]) and up to three other anti-glycan antibodies concurrently. Serum levels of anti-glycan antibodies in CF and CD were not related to parameters of inflammation. Despite the well-documented difference in clinical course between male and female CF patients no gender difference of anti-glycan antibodies was found. In contrast, there was a significant positive correlation between anti-glycan markers and age in CF patients. Conclusions: Our findings demonstrate for the first time the increased frequency of a panel of anti-glycan antibodies in CF and provide a link between the presence of these serological biomarkers and patient's age. Anti-glycan antibody profiling may therefore become a valuable tool in the care of patients with CF.


Background: Erythropoietin, originally recognized for its role in erythropoiesis, has been shown to improve neurological outcome after stroke. Low-dose methotrexate is effective against certain inflammatory diseases, such as severe psoriasis and rheumatoid arthritis as well as Crohn's disease. Immunosuppressive effect of methotrexate also reduces the proportion of patients with chronic progressive multiple sclerosis with modest clinical benefits. Combination of erythropoietin and methotrexate can target neuroinflammation along with immunosupression. Objective: To evaluate the role of erythropoietin and methotrexate in experimental autoimmune encephalomyelitis, a commonly used animal model of several degenerative human diseases like multiple sclerosis. Materials and Methods: In the present study, C57BL/J6 mice were immunized with 200 mu g of myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA) supplemented with 1 mg/ml of killed mycobacterium tuberculosis (MBP: CFA in 1: 1 ratio). These animals were given a combination of methotrexate and erythropoietin. Neurological function tests were scored daily by grading of clinical signs. Cerebral histopathology was performed to detect inflammatory infiltrates and demyelination. Results: Treatment with erythropoietin and methotrexate significantly improved the neurological function recovery, reduced inflammatory infiltrates, and demyelination as compared to controls possibly by stimulating oligodendrogenesis and down-regulating proinflammatory infiltrates. Conclusion: The findings suggest an adjunctive use of methotrexate in demyelinating disease.