Rhodococcus equi is a primary pathogen of the lower respiratory tract of foals and a saprophyte thriving in the intestine of horses and manure-containing farm soils. This dual lifestyle makes this bacterium both a significant burden to the thoroughbred industry and an interesting model to study niche adaptation. Infection of alveolar macrophages often leads to pyogranulomatous cavitating pneumonia. Rhodococcus equi withstands phagocytosis by diverting the phagolysosomal pathway into vacuoles permissive for bacterial proliferation. Disruption of the phagolysosome biogenesis depends on the presence of a pathogenicity island (PAI) located within a conjugative plasmid. The PAI encodes a family of six virulence associated proteins (Vap), which are unique to Rhodococcus equi. Among them, VapA is the only cell surface-located member of the family and, until recently, the only PAI gene with a clearly identified role in virulence: arrest of phagosome maturation [1]. Our recent work has shown two other virulence-associated functions of the PAI: modulation of the intracellular growth by IcgA [2] and co-option of the chromosomal gene expression by the PAI transcriptional regulators VirR and VirS [3]. IcgA is a member of the major facilitator superfamily transport proteins and its deletion resulted in a hypervirulent phenotype. VirR and VirS regulate the expression of vapA and other PAI genes in response to environmental cues associated with the host environment. In addition, transcriptomic analysis revealed that their regulatory effect spans over 20% of Rhodococcus equi transcriptome, resulting in significant changes of transport processes, energy production and cellular metabolism. In summary, the three known functions of the PAI are: i) VapA creates an intracellular niche for Rhodococcus equi, ii) IcgA helps maintaining the niche created by VapA and iii) the concerted regulatory effect of VirR and VirS on the chromosomal gene expression adjusts the bacterial physiology to suit the requirements in the host environment. Interestingly, genomic evidence strongly indicates that the PAI was acquired by the avirulent ancestor of Rhodococcus equi in an event of lateral gene transfer from a yet unknown donor [4] and most chromosomal virulence-associated genes are conserved in non-pathogenic Actinobacteria [5]. An example of the above is the recruitment of the highly conserved biosynthetic genes for the siderophore Rhequichelin for iron acquisition in the host environment [6].

Taken together, these data indicate that the emergence of virulence in a non-pathogenic ancestral bacterium was a gradual process that was initiated by the acquisition of key adaptation genes within the PAI, followed by alteration of chromosomal gene expression patterns resulting in an adjustment of bacterial physiology to suit the environment of the infected host.

References


064 Novel Determinant Confers Macrolide, Lincosamides, and Streptogramin B resistance in Rhodococcus equi

Londa J. Berghaus*,1, Steeve Gigueré*1, Elisa Anastasi2, Mary K. Hondalus3, Jennifer M. Willingham-Lane4, Iain MacArthur5, Noah D. Cohen4, Marilyn C. Roberts3, Jose A. Vazquez-Boland1,6

1 Department of Large Animal Medicine and; 2 Department of Infectious Diseases, University of Georgia, Athens, Georgia, USA; 3 Microbial Pathogenesis Unit, School of Biomedical Sciences and The Roslin Institute, University of Edinburgh, Edinburgh, UK; 4 Department of Large Animal Clinical Sciences, Texas A&M University, College Station, Texas, USA; 5 Department of Environmental and Occupational Health Sciences, School of Public Health University of Washington, Seattle, Washington, USA; 6 Grupo de Patogenómica Bacteriana, Facultad de Veterinaria, Universidad de Léon, Léon, Spain

The incidence of macrolide and rifampin resistance in R. equi isolated from foals has increased considerably in recent years. The objective of this study was to identify the molecular mechanism of emerging macrolide resistance in R. equi and to determine its transferability. Macrolide-resistant (n=62) and macrolide susceptible (n=62) clinical isolates of R. equi from foals in the USA were studied. Whole genome sequencing of a sample of 18 macrolide-resistant and 6 macrolide-susceptible R. equi was performed. PCR was used to screen for the presence of the resistance determinant in the other isolates. Mating experiments were performed to document transfer of the determinant. A novel erm gene, erm(46) was identified only in resistant isolates. There was perfect association between macrolide resistance and...
presence of erm(46) as detected by PCR in 124 isolates of R. equi. Expression of erm(46) in a macrolide-susceptible strain of R. equi induced high level resistance to macrolides, lincosamides, and streptogramins B, but not to other classes of antimicrobial agents. Transfer of erm(46) from resistant to susceptible strains of R. equi was confirmed and occurred at a transfer frequency of up to $2 \times 10^{-3}$. This is the first molecular characterization of macrolide, lincosamides and streptogramins B resistance in R. equi. Resistance is caused by a novel erm gene, erm(46), which is transferrable likely by conjugation.

071 Chloroquine inhibits Rhodococcus equi multiplication in murine (J774A.1) and foal alveolar macrophages

L.T. Gressler*1, A.J. Bordin 2, C.M. McQueen 2, A.C. de Vargas 1, N.D. Cohen 2
1 Laboratory of Bacteriology, Department of Preventive Veterinary Medicine, Federal University of Santa Maria, Santa Maria, RS 97105-900, Brazil; 2 Equine Infectious Disease Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77845, United States

Rhodococcus equi is a facultative intracellular pathogen that primarily infects macrophages causing pyogranulomatous pneumonia and many other extrapulmonary disorders in young foals. The ability of R. equi to multiply in alveolar macrophages (AMs) coupled with increasing prevalence of antimicrobial resistance are the major obstacles for disease control. There is a consensus that iron is a micronutrient essential for all microorganisms; however, its availability also influences intracellular replication and expression of virulence genes of R. equi. Control of iron availability by host cells is a component of nutritional immunity in vertebrates. Similarly, the drug chloroquine (CQ), an aminquinoline, has been used against intracellular pathogens to limit iron availability inside phagocytic cells. Our hypothesis was that CQ would suppress the growth of R. equi inside macrophages. We evaluated the R. equi killing capacity of murine (J774A.1) and foal AMs exposed to CQ (incubated 24 h prior to infection) with or without saturated transferrin (HTF). HTF prevents the accumulation of iron in cells, whereas HTF is a source of iron to R. equi, enhancing the bacterium ability to survive and replicate intracellularly. Thus, we investigated whether HTF would revert the inhibition by CQ of intracellular survival of R. equi. We observed a significant (P < 0.05) inhibition of R. equi proliferation in both murine J774A1 and foal AMs exposed to 10 and 20 μM CQ for 24, 48, and 72 hours post-infection. HTF (6 mg/ml) did not reverse CQ inhibition of R. equi intracellular survival, suggesting that CQ impairs the iron uptake by R. equi cells via HTF, as observed in other intracellular pathogens, such as Legionella pneumophila and Paracoccidioides brasilensis. We conclude that CQ suppresses R. equi growth in both murine J774A1 and foal AMs. Further research is warranted to confirm its purported mechanism of iron-deprivation, as well as its therapeutic potential against R. equi infection of foals in vivo.

Acknowledgements

The authors acknowledge the scholarship (process 202217/2014-0/SWE) supplied by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)" to Leticia T Gressler. Additional support was provided by the Link Equine Research Endowment, Texas A&M University.

072 Oxidative stress and Rhodococcus equi pneumonia

J. Crowley, J. Fishburn, E. Po, P. Celi, G. Muscatello* Faculty of Veterinary Science, University of Sydney, New South Wales, 2006

Oxidative stress (OS) is defined as an imbalance between oxidants and antioxidants. OS has been shown to play roles in various equine respiratory diseases, specifically in association with various inflammatory airway conditions. The role of OS with respect to infectious respiratory diseases of horses has been relatively poorly explored. The significant of OS in the pathogenesis Rhodococcus equi pneumonia in foals is unknown. The objective of these studies was to measure and relate respiratory and systemic biomarkers of OS to the pathology of R. equi pneumonia and to the risk of developing this disease. An initial case-control study compared various OS biomarkers from blood and exhaled breath condensate (EBC) samples collected from 26 foals (n=12, cases and n=14, controls) residing on farms endemic for the disease. Foals were defined as cases (positive) or controls (negative) based on ultrasonographic evidence of pulmonary abscessation (>15 mm in diameter). Haematology testing was also performed on bloods collected. Comparison of biomarkers between the groups was performed using two-sample t-tests. The following season a prospective case-control study was performed on 74 foals (n=27, cases and n=47 controls) in which systemic OS was evaluated from blood samples collected within 24 hours of birth and related to future diagnosis of R. equi pneumonia as defined in the previous study. The initial case-control study showed reactive oxygen metabolites in blood (d-ROMs) to be significantly greater in case foals (P=0.027) whilst the oxidative stress index (OSI) was also highest in case foals (P=0.014). Hydrogen peroxide (H2O2) concentrations in EBC was also significantly greater in cases (P=0.002). In the prospective case-control study, foals that developed disease had a significantly lower biological antioxidant potential in blood (P=0.049) within 24 hours of birth compared to those which didn't develop disease. These findings suggest that OS plays a role in the pathogenesis of R. equi pneumonia and antioxidant capacity may contribute to the innate risk of disease development in the neonatal foal. These findings open up the potential use of OS biomarker assays in the diagnosis of R. equi pneumonia and potentially the identification of 'at risk' foals at birth. These results also open up the potential application of antioxidant therapeutics to aid in the clinical management of foals with R. equi pneumonia and as a non-antimicrobial prophylactic in reducing risk of the neonatal foal developing disease.

Bibliography