

Improvement of genome annotation for bovine respiratory disease pathogens by proteogenomic mapping. Mark L Lawrence¹*, James Watt¹, Susan Bridges², Bindu Nanduni¹, Nan Wang², Ranjit Kumar¹, and Shane C Burgess¹. ¹College of Veterinary Medicine, Mississippi State University, Mississippi State, MS. ²Department of Computer Sciences and Engineering, Mississippi State University, Mississippi State, MS

High throughput proteogenomic mapping was conducted to provide economic, rapid, and comprehensive experimental evidence to improve genome annotation of the three bovine respiratory disease (BRD) pathogens *Mannheimia haemolytica*, *Histophilus somni*, and *Pasteurella multocida*. Another objective of this project was to improve annotation tools available for researchers to conduct functional genomics and systems biology investigations on these pathogens. Proteins were isolated from *M. haemolytica* strain PHL213, *P. multocida* strain 3480, and *H. somni* strain 2336 in triplicate and analyzed by multi-dimensional protein identification technology (MuDPIT) using two-dimensional liquid chromatography with electrospray ionization tandem mass spectrometry. The resulting mass spectra were searched against their respective protein databases using SEQUEST (Bioworks 3.2 cluster, ThermoElectron). For proteogenomic mapping, tandem mass spectra were also searched against the respective genome sequences translated in all six potential frames using SEQUEST. For peptides only identified from the genome sequence, our automated proteogenomic mapping pipeline was used to produce expressed protein sequence tags (ePSTs), which are the theoretical protein coding sequences that contain the peptide sequence identified by mass spectrometry. Biological evidence was then incorporated to allow evaluation of the strength of each ePST. As a result of this project, experimental evidence is being provided for the existence of annotated protein products, and proteins are being identified that were not predicted in the genome annotation. This information will directly improve the genome annotations and will result in a better understanding of the size and diversity of proteomes in *Pasteurellaceae*.

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Establishment of Bovine Primary Respiratory Epithelial Cell Cultures From Live Calves and Evaluation of Cell Responses to Bovine Respiratory Syncytial Virus Infection. T M Krunkosky¹, C L Jarrett¹, M B Ard², C L Betts³, L J Berghaus³, K A E Hurley³, A R Woolums³. ¹Dept of Anatomy and Radiology, ²Dept of Pathology, ³Dept of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA

Little is known about the influence of bovine respiratory epithelial cells (BREC) on immune responses to infectious bovine respiratory disease. Bovine respiratory syncytial virus (BRSV) is a major contributor to bovine respiratory disease. An *in vitro* BREC model that can be frozen and passaged is needed to evaluate the influence of BREC on cellular immune responses to agents such as BRSV. BREC were isolated by bronchial brushing from 2-week-old calves. Cells were collected from secondary or tertiary bronchi. After culture, de-differentiated cells (classified morphologically by lack of cilia and squamous appearance) were frozen as passage 1. Thawed undifferentiated cells were then grown as passage 2 in an air-liquid interface culture system to a differentiated phenotype. Differentiated primary BREC were evaluated by electron microscopy, confirming the presence of confluent monolayers of ciliated and nonciliated cells, including goblet cells. Confocal microscopy was also utilized to characterize cellular morphology and identify cilia and F-actin distribution. Prior to full differentiation cells were infected with BRSV; infection was confirmed by immunofluorescence. Expression of mRNA for the chemokines IL-8 and RANTES and the adhesion molecule ICAM-1 by infected cells was evaluated by real time RT-PCR, at 2 hours postinfection (PI). IL-8 and ICAM-1 mRNA were significantly increased. Levels returned to baseline at 4 and 24 hours PI. *In vitro* culture of passaged respiratory epithelial cells from live calves will enable evaluation of the cellular immune responses to BRSV and other infective agents, including MHC-restricted interactions between epithelial cells and T cells.

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Effects of bovine herpesvirus type 1 infection of bovine bronchial epithelial cells on neutrophil adhesion and activation. José J Rivera-Rivas¹ and Charles J Czuprynski². ¹Department of Bacteriology and ²Department of Pathobiological Sciences, School of Veterinary Medicine University of Wisconsin-Madison

Bovine Herpesvirus type 1 (BHV-1) is an important viral agent in the bovine respiratory disease (BRD) complex. BHV-1 infection results in recruitment and activation of various cell types in the airways. One cell type that has received limited investigation in response to BHV-1 infection is the bovine bronchial epithelial (BBE) cell. In the present study we investigated BHV-1 infection of BBE cells and what effect that might have on neutrophil adhesion and activation. *In vitro* BBE cells were infected with BHV-1 and 1 to 24 hr post infection BBE cell RNA was extracted, transcribed to cDNA, and cytokine expression assessed by real-time PCR. We found that BHV-1 infection elicits a rapid IL-1, IL-8 and TNF- α mRNA response by BBE cells. Bovine peripheral blood neutrophils (PMNs) exhibited greater adherence to BHV-1 infected BBE cells than uninfected cells. The increased adherence was significantly reduced by the addition of an anti-IL-1 β antibody. We also found that conditioned media from BHV-1 infected BBE cells induced shape change and degranulation in bovine PMNs, and increased their LFA-1 expression. The latter in turn was associated with increased susceptibility of the PMNs to *Mannheimia haemolytica* LKT. Our results suggest that BHV-1 infection of BBE cells triggers cytokine expression that contributes to activation and attachment of neutrophils, and amplifies the detrimental effects of *M. haemolytica* on these cells. These events could contribute to the intense pulmonary inflammation that characterizes BRD.

Nitazoxanide and tizoxanide inhibit animal respiratory virus replication *in vitro*. L Ashton^{*}, LS Goehring, GA Landolt, and RJ Callan. Colorado State University College of Veterinary Medicine and Biological Sciences, Fort Collins, CO

There is a need for development of specific antiviral treatment of respiratory viral diseases including equine and bovine herpes virus type 1 (EHV-1, BHV-1), equine, canine, and swine influenza (EIV, CIV, SIV), bovine viral diarrhoea virus (BVDV type I & II), bovine parainfluenza virus type 3 (PI-3), and bovine respiratory syncytial virus (BRSV). Nitazoxanide (NTZ) and its metabolite, tizoxanide (TIZ), are thiazolidine compounds that demonstrate antimicrobial activity against a variety of protozoal, bacterial, and viral organisms. In this study, the ability of NTZ and TIZ to decrease replication of common respiratory viruses including EHV-1, EIV, CIV, SIV, PI-3, BRSV, BVDV, BHV-1 was evaluated by determining the antiviral effective concentrations that result in 50% (EC₅₀) or 90% (EC₉₀) reduction of virus production in cell culture supernatants. Isolates of EHV-1, EIV, CIV, SIV, BRSV, BHV-1, PI-3, and BVDV were cultured on cells susceptible to infection at 0.0001 MOI in the presence of varying concentrations of NTZ and TIZ for 96 hr. Viral titers of culture supernatants were determined by plaque assay or TCID₅₀. Viral titer of culture supernatants infected with BRSV were determined at 168 hr by TCID₅₀. The EC₅₀ and EC₉₀ for the compounds were determined by polynomial regression of viral titers. The EC₅₀ and EC₉₀ were not significantly ($p > 0.05$) different between NTZ and its metabolite TIZ for all viruses tested except EHV-1. Both compounds were most effective at inhibiting replication of influenza A (EIV, CIV, and SIV, EC₅₀ 0.13-0.29 μ M), followed by herpesvirus 1 (EHV-1 and BHV-1, EC₅₀ 0.65-0.96 μ M), PI-3 (EC₅₀ 1.48-1.72 μ M), BVDV (EC₅₀ 1.48-2.47 μ M), and BRSV (EC₅₀ 2.07-2.43 μ M). The EC₉₀ values are approximately 3.5 times higher than the EC₅₀ for all viruses tested. The *in vitro* results indicate that NTZ may be an effective drug for the prevention or treatment of viral respiratory infection in animals. Based on these results, further *in vivo* testing of NTZ for the control or treatment of viral respiratory diseases affecting animals is warranted.

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The Irish animal traceability system: A descriptive analysis of bovine tuberculosis reactor animals during the year 2006. F J Olea-Popelka¹, P White², G McGrath³, J O'Keefe², SW Martin³. ¹Department of Clinical Sciences, Colorado State University, Fort Collins, CO USA. ²Department of Agriculture and Food, Ireland. ³Department of Population Medicine, University of Guelph, Ontario, Canada

In Ireland, as part of the ongoing bovine tuberculosis (BTB) eradication scheme, every herd (animal) is tested at least once a year using the single intradermal comparative tuberculin test (SICCT). The objective of our study was to use the available data in order to create a computerized code that will allow, in a standardized manner, describe the BTB related events on a year basis, both at the animal and the herd level. Two national computerized databases were used for our study: (1) the Animal Health Computer System and (2) the Cattle Movement and Monitoring System. We identified all the BTB reactor animals during the year 2006 and traced them back to their herd of origin. During the year 2006, 24,188 animals were classified as BTB reactors in Ireland. Sixty four percent of these animals were homebred (i.e. they never moved between herds), while the remaining 36% moved between two or more different herds. Thirty six percent of the homebred BTB reactor animals had evidence of previously being present in the herd during a previous BTB episode (before the year 2006). The median age of the BTB reactor animals was 3.1 years. In each county, an age ratio was calculated and compared to the rest of the country. The proportion of BTB "standard reactors" was obtained for each county and compared to the national average as a measure of the severity of the eradication scheme. A more severe interpretation of the SICCT was applied in county Louth. The computer code created for this analysis will allow descriptions on the characteristics of the BTB reactor animals and herds involved on a yearly basis. This exercise will become an important tool in describing the progress of the bovine tuberculosis eradication scheme in Ireland.

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Removal of endotoxin from dairy wastewater. Charles W Purdy^{*}, USDA-ARS, Bushland, TX. David C. Straus, Texas Tech University Health Sciences Center, Lubbock, TX.

The efficacy of various treatments on removing endotoxin (ET) from wastewater was tested by using the treated water to induce a systemic reaction via intratracheal inoculation (20 ml/goat, 6 goats/group). Treatments (T1-T7) of wastewater were as follows: 1) autoclaved 15 min, centrifuged and contained 17.56 μ g ET/20 ml. 2) treated once with calcium silicate hydrate (LRA) (15g/L), centrifuged, sterile filtered, contained 0.962 μ g ET/20 ml. 3) treated once with LRA, centrifuged, sterile filtered and ET replaced with known E. coli ET, contained 16.60 μ g/20 ml. 4) treated 5 times with LRA, autoclaved 10 times, frozen and thawed 3 times, centrifuged, sterile filtered, contained 3.12 μ g ET/20 ml. 5) treated 5 times with LRA, centrifuged, sterile filtered, contained 2.72 μ g ET/20 ml. 6) Sterile ET free water. 7) Pen controls, no treatments. Systemic reactions were monitored by rectal temperatures (RT) and total white blood cell (WBC) counts. Results were as follows: T1 and T3 were not significantly different and gave a typical ET systemic response (> rectal temperature and total WBC). T6 and T7 had no ET temperature response. T2 and T5 had shorter (>3-4 hr RT) ET response, and T4 was similar to the T6 and T7 RT response, except for slight increases in RT at 6 and 12 hrs. These results suggest an ET dose of 0.9 to 3.12 μ g induced an aberrant *in vivo* systemic response.



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