

Diversity of *Salmonella* spp and other pathogens in dairy wastewater flow on the Southern High Plains. Charles W. Purdy*, USDA-ARS, Bushland, TX, R. H. Raleigh, Veterinary Diagnostic Laboratory, Texas A&M University, Amarillo, TX, David C. Straus, Texas Tech University Health Sciences Center, Lubbock, TX.

Wastewater flows of 4 large (2,500 to 4,500 head) dairies were investigated for potential zoonotic pathogens. Study objectives were to quantify the microbes and identify pathogens along the dairies wastewater flows. Wastewater started with an automatic wash of the milking parlor floor, and then flowed through open channels or drainage pipes to the lagoon. Lagoon water was pumped to center-pivot irrigation pipes for irrigation of forage crops. Three of the 4 dairies used recirculated lagoon water to periodically flush behind the feeding alleys to move manure back into the lagoon. Wastewater was collected in triplicate from 13 sites along the dairy water flows for microbial isolation. General and selective media and enrichment broths were used to grow and identify the microbes. The Biolog identification system was also used to identify bacteria and yeast. Results: Sixteen *Salmonella enterica* serovars among 478 *Salmonella* isolates, *Listeria monocytogenes*, *L. (Jonesia) denitrificans*, *Providencia alcalifaciens*, *Streptococcus hyointestinalis* Group B, *Escherichia fergusonii*, *E. coli* O157:H7, *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, and *Bordetella bronchiseptica* were isolated. Conclusions: Pathogens identified in dairy wastewater indicated that caution is necessary when using this water in order to prevent the spread of zoonotic pathogens on the dairy, and to prevent the infection of human and bovine hosts.

Potential impact of foot-and-mouth disease incursions in the High Plains region of Texas. M.P. Ward¹*, L.D. Highfield¹, M.G. Garner². ¹Dept. of Veterinary Integrative Biosciences, Texas A&M University, College Station TX, U.S.A. ²Department of Agriculture, Forestry and Fisheries, Canberra ACT, Australia

A stochastic, state-transition simulation model was used to investigate the potential impact of foot-and-mouth disease incursions in the High Plains region of Texas. The study region is an 8-county area of northwest Texas, containing >2 million cattle on-feed and approximately 400,000 grazing cattle in >10,000 herds. A previously developed model was updated with regional-specific herd types, estimated contact rates and estimates for key biologic parameters, based on expert opinion. A range of incursion scenarios (backyard, small feedlot, large feedlot, commercial beef) and mitigation strategies (slaughter of infected and dangerous contact herds, with or without ring vaccination, with or without adequate vaccine availability, early or late detection of the outbreaks, enhanced disease surveillance) were simulated. The predicted model outbreaks are being analyzed using a multivariate analysis of variance design. Preliminary results suggest that when an outbreak is detected (early versus late) has the greatest impact on the likely outcome of the incursion. This epidemic simulation model is currently being incorporated into a decision support system.

Regional survey of animal shelters on infection control policies and zoonotic disease awareness. K. Steneroden*, A. Hill. Animal Population Health Institute, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Animal shelter animals and workers are a potentially vulnerable population whose level of exposure to zoonotic diseases is largely unknown. The objective of this project was to determine the level of infection control and zoonotic disease awareness practiced by animal shelters in Colorado, Wyoming, Utah, Montana, North Dakota and South Dakota and compare shelters by size, shelter type and location. A needs assessment survey was developed and mailed to 157 animal shelters. Survey questions focused on shelter demographics, infection control practices and policies, awareness and concern over infectious and zoonotic diseases, estimated levels of infectious and zoonotic disease, staff and volunteer training relating to infection control and zoonotic disease awareness, utilization of diagnostic tools, isolation procedures and protocols, and communication with the public. We received a 50% response rate from a wide variety of shelter types, sizes and locations. Infectious diseases of greatest concern to shelters include feline upper respiratory disease, canine parvovirus and feline panleukopenia. Zoonotic diseases of greatest concern include ringworm, and fecal parasites. Zoonotic diseases of slight or no concern include plague, tularemia and leptospirosis. Approximately 25% of shelter staff and volunteers receive no training in infection control principles and practices. Approximately 30% receive no training in infectious disease identification and even greater percentage (up to 50%) receives no training in zoonotic disease identification. Overall volunteers receive less training in these areas than staff members. Ninety percent of shelters said they would benefit from training in infectious and zoonotic disease. Animal shelters in our six state area may benefit from training particularly in infection control practices, zoonotic disease awareness and cleaning and disinfection.

Development of a one-step real time reverse transcription PCR assay for the rapid detection of canine influenza virus in nasal swab specimens. M.E. Spindel¹*, S. Dillion¹, K.F. Lunn¹, G.A. Landolt¹. Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Canine influenza virus (CIV) was first isolated from dogs in 2004. The virus has spread rapidly in the United States' dog population since then. Nearly one hundred percent of dogs exposed to CIV become infected as most dogs have no natural or vaccine induced immunity to the antigen. Thus, rapid and accurate diagnosis of CIV infection is critical in the control of the disease. Real-time reverse transcription (RT)-PCR based assays have been shown to be highly sensitive and specific diagnostic tools. This project's aim was establishing a RT-PCR assay for the detection of CIV. In general, RT-PCR is performed by first converting RNA to cDNA during a separate RT reaction, followed by a standard PCR reaction. As sample handling increases the risk of cross-contamination and carryover, we developed a one-tube real-time RT-PCR for the detection of CIV. Primers and probe were chosen to detect a highly conserved region in the matrix (M) gene. The sensitivity and specificity of the assay was evaluated using an M gene RNA standard, as well as dilution series of four H3N8 influenza virus strains. 105 nasal swab samples collected from dogs with clinical signs of respiratory disease were tested to evaluate the assay's performance. The results of the real-time RT-PCR assay were compared with results of virus isolation in Madin Darby canine kidney (MDCK) cells and embryonated chicken eggs, detection of influenza virus nucleoprotein (NP) using a commercially available optical immunoassay (OIA) kit (Directigen Flu A®D, Becton Dickinson, Franklin Lakes, NJ), and to serology. Our results demonstrate that real-time RT-PCR was more sensitive in the diagnosis of CIV in dogs demonstrating clinical signs of respiratory disease than virus isolation, OIA, and serology. In conclusion, the real-time RT-PCR assay is an excellent tool for improving the early and rapid detection of CIV during outbreaks of respiratory disease.

Genetic characterization of H3N8 influenza viruses isolated from dogs and horses in Colorado and Wyoming between 2006 and 2007. S. Dillion¹, M. E. Spindel¹, and G. A. Landolt¹* ¹Dept. of Clinical Sci., College of Vet. Med. and Biomed. Sci., Colorado State University, Fort Collins, CO

Since spring 2004 canine influenza virus has caused outbreaks of respiratory disease in dogs in many parts of the United States. The appearance and spread of the virus throughout the American dog population constitutes a dramatic epidemiological shift, since dogs have not been regarded as hosts for influenza A viruses. Although influenza viruses are occasionally transmitted from one host species to another, such transmission events tend to be self-limiting and newly introduced viruses are only rarely maintained in a new host species. In this regard, it has been suggested that persistence of a virus in another species requires mutational adaptation to the new host. As the hemagglutinin (HA) protein is considered to be a critical determinant of species specificity of influenza, we sequenced the full length protein coding regions of the HA as well as neuraminidase (NA) genes from six H3N8 viruses recovered from dogs in Colorado and Wyoming between May 2006 and April 2007, and four H3N8 viruses isolated from horses in Colorado in 2006 and 2007. Phylogenetic analyses revealed that the canine H3N8 isolates have maintained several amino acid differences in the HA protein (including I29M, N54K, N83S, L118V, W222L, I328T, and G479E), when compared to recent equine isolates. In contrast to previously published data (Crawford *et al.* 2005.Science. 310:482-485), the amino acid substitution (N>T) at residue 483 has not been maintained in the most recent canine isolates recovered in Colorado. The fact that the canine influenza viruses have spread widely in the dog population of the United States and have strictly maintained seven amino acid substitutions, suggests that these mutations may represent dog adaptation mutations.

Molecular typing of Influenza A viruses using gel-based biochip technology. Kireev D.E., Grebennikova T.V., ZaberezhnyA.D*., Aliper T.I., Nepoklonov E.A. D.I.Ivanovski Virology Institute, Moscow, Russia.

Influenza virus has become endemic in a number of species, including humans, swine, horses, and poultry. Typing of the isolated viruses has predictive value for prevention and control strategies. We routinely use previously developed PCR and real time PCR to detect the virus and identify H5, N1 and H7 subtypes. In addition we use blocking ELISA to screen the blood serum for virus-specific antibodies in wide range of species. Recent outbreaks of Influenza A H5N1 in poultry lead us to regular screening of poultry, livestock and wild animals. For this purposes a biochip was developed containing 3D gel-based elements with the designed set of discriminative oligonucleotides. The technology was provided by E.E.Fesenko at Biochip-IMB Company based at V.A. Engelhardt Institute of Molecular Biology (Moscow) on collaborative basis. The procedure includes two-stage multiplex amplification of the HA and NA gene fragments followed by on-chip hybridization of the PCR products. The method enables us to identify 15 subtypes of HA (H1-H15) and N1, N2 subtypes of NA, allowing accurate recognition of both human (H1N1, H3N2) and avian (H9N2, H5N1) subtypes within 10 hours. The biochip was validated with the panel of 18 known strains of influenza A virus. The successful subtype identification was demonstrated using the nasopharyngeal swabs taken from humans (H3N2) and field samples taken from dead birds during epizootic (H5N1).



PROCEEDINGS

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December 2, 3 and 4, 2007
Marriott, Downtown Magnificent Mile
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