and 7 had received the 4-way. Of the 28 dogs with vaccination history, 14 had been vaccinated within the preceding 6 months. Only one (4-way) had a titer ≥200 for the combination of *canicola* and *icterohemmorghiae*, without seropositivity to the non-vaccinate serovars. Seropositivity to *bratislava*, not included in any of the vaccines available, was present in 10 dogs. Of the seronegative dogs, 20% (81/414) are known to have been previously vaccinated.

This study demonstrates a seroprevalence of 14% for *Leptospira* in Midwest dogs. Most commonly detected were *grippotyphosa* and *bratislava*, which correlates with data from another investigation. Antibodies were detected in all age groups. Vaccination status was known in 41% of the seropositive dogs. Patterns of titers in vaccinates were difficult to interpret due to either lack of antibody response to some components of the vaccine, or to antibodies against non-vaccinate serovars.

195 ATTEMPTED TRANSMISSION OF *MYCOPLASMA HAEMOFELIS* BY INGESTION OF *M. HAEMOFELIS*-INFECTED FLEAS.

JE Woods, MR Lappin, N Wisnewski. Department of Clinical Sciences (Woods, Lappin), Colorado State University, Fort Collins, CO; Heska Corporation (Wisnewski), Fort Collins CO.

Feline infectious anemia is caused by at least two *Mycoplasma* species; *Mycoplasma haemofelis* and '*Candidatus* M. haemominutum'. Although experimentally the organisms have been successfully transferred by a variety of routes, all natural modes of transmission for these organisms have yet to be elucidated. Blood-sucking arthropods have long been incriminated in the natural transmission of the disease, and recently, our experiments have shown that the cat flea, *Ctenocephalides felis*, can transfer *M. haemofelis* between cats during hematophagous activity. In those studies, the cats were unable to groom and ingest the fleas or flea excrement, a significant activity of cats with natural flea infestations. We hypothesize that the cat flea may transmit *M. haemofelis* when infected fleas or flea by-products are ingested, a transmission route known in other infectious diseases (e.g. *Dipylidium caninum*). The goal of this study was to determine whether *M. haemofelis* infection of previously naïve cats could be initiated by the ingestion of infected fleas.

Four, young-adult, mixed-sexed cats were used. Two cats were known chronic carriers of *M. haemofelis*. The other two cats were shown to be negative for hemoplasmosis by a PCR assay that amplifies the DNA of both *Mycoplasma* species. One flea chamber containing 100 *C. felis* fleas was attached to each of the chronic carrier cats and left in place for a period of five days during which time the fleas could feed. At chamber removal, a random sample of fleas was analyzed by PCR assay and shown to be positive for *M. haemofelis* DNA. The remaining fleas and flea by-products were then fed to each of the two *M. haemofelis*-naïve cats. One cat was fed 91 viable fleas (37 female, 54 male) and 0.2 gram of flea by-products, including feces, larvae and eggs. The other cat was fed 93 viable fleas (39 female, 54 male) and 0.163 gram of flea by-products. These items were mixed into 71 grams of a commercially available beef-based human baby food to facilitate feeding.

A CBC and PCR assay performed weekly failed to document infection during the first six weeks post-oral inoculation. Results of this study suggest that ingestion of *M. haemofelis*-infected fleas is not be a route of transmission, an inadequate quantity of fleas was fed, the timing of flea feeding was inappropriate for transmission, or the observation time was inadequate.

196 DISCRIMINANT ANALYSIS OF EXTENDED UROVIRULENCE GENOTYPES DISTINGUISHES HUMAN, CANINE, AND FELINE URINARY *ESCHERICHIA COLI* ISOLATES FROM NEW ZEALAND.

T. Freitag, R.A. Squires. Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, NZ.

Numerous genes of *E. coli* encode proteins putatively important to urovirulence, for example, adhesins and protectins. Human, canine, and feline urinary *E. coli* isolates have been characterized on the basis of their extended urovirulence genotypes in studies that typically test for the presence or absence of about 25 of these genes. It has been reported recently that extended urovirulence genotypes of canine and feline urinary *E. coli* isolates overlap with, and are essentially indistinguishable from, those of human strains that cause serious extraintestinal infections. On the basis of these and other phylogenetic findings, concern has been expressed that some canine and feline uropathogenic *E. coli* strains pose a significant human health hazard. However, very few canine isolates and even fewer feline isolates have been adequately studied to date.

We investigated whether discriminant analysis could accurately classify a urinary *E. coli* isolate as originating from a dog, cat, or human based on its extended urovirulence genotype. This genotype was obtained by multiplex PCR analysis of 25 putative urovirulence genes in each isolate. 45 canine and 22 feline urinary *E. coli* isolates were acquired from a large network of veterinary clinical pathology laboratories in New Zealand. 11 human isolates were obtained from the clinical microbiology laboratory of a local hospital. All isolates were acquired between November 2001 and November 2003, originating from cases undergoing clinical investigation of suspected urinary tract inflammation and/or infection. Statistical analysis was carried out using the DISCRIMINANT function of SPSS ver. 11.5, with cross-validation, using a stepwise procedure based on minimization of Wilk's lambda.

When the genotypes of the feline and human isolates were subjected to discriminant analysis, 21/22 (96%) of the feline isolates and 10/11 (91%) of the human isolates were correctly classified. Analysis of genotypes from canine and human isolates resulted in a correct categorization of 43/45 (96%) of the canine isolates and 7/11 (64%) of the human isolates. In a combined analysis of all three populations' genotypes only 4/45 (9%) of the canine isolates and 1/22 (5%) of the feline isolates were misclassified as being of human origin. When analyzing the genotypes of human isolates versus a combined set of companion animal genotypes, 63/67 (94%) of the non-human isolates and 8/11 (73%) human isolates were correctly classified.

Contrary to previous findings, these preliminary results suggest that it may be possible to differentiate canine and feline urinary *E. coli* isolates from human isolates with reasonable accuracy using discriminant analysis of urovirulence genotypes. These results will be of interest to researchers studying comparative aspects of *E. coli* urovirulence and others seeking to determine which, if any, canine and feline urinary *E. coli* genotypes are of particular zoonotic concern.

197 PREVALENCE OF FHV-1, *MYCOPLASMA* SPP., AND AEROBIC BACTERIA IN SHELTER CATS WITH ACUTE UPPER RESPIRATORY TRACT DISEASE.

JK Veir,[a] R Ruch-Gallie,[a] ME Spindel,[b] and MR Lappin.[a] From the [a]College of Veterinary Medicine and Biomedical Sciences, Colorado State University and [b]Larimer Humane Society; Fort Collins, CO.

Infectious causes of upper respiratory disease in cats are common in humane societies. Feline herpesvirus 1 (FHV-1) is thought to be the most common infection. With the exception of *Bordetella bronchiseptica* and *Chlamydophila felis*, most bacterial infections are thought to be secondary to other primary diseases. *Mycoplasma* spp. are known to be normal oropharyngeal flora in some cats but have also been proposed to be associated with feline upper respiratory tract disease. The purpose of this study was to describe the isolation rates of FHV-1, aerobic bacteria, and *Mycoplasma* spp. from cats with acute clinical upper respiratory disease in a humane society in North Central Colorado.

Between January 24, 2003 and December 4, 2003, 61 cats with clinical evidence of acute upper respiratory disease had swabs collected from the right nares and right pharyngeal region. The first swab was placed in transport media and submitted within 4 hours for aerobic bacterial and *Mycoplasma* spp. culture. A second swab for DNA extraction from each site was placed in sterile saline and stored at −80°C between 2 and 3 hours after collection until batch analysis. Fluorogenic PCR targeting FHV-1 was performed on the DNA extracts.

The majority of cats with positive results were positive on both nasal and pharyngeal swabs and so results from the 2 sites were combined. Of the 61 cats, DNA of FHV-1 was amplified from 52 cats, aerobic bacteria were cultured from 57 cats, and *Mycoplasm*a spp. were cultured from 35 cats. The distribution of positive results were as follows: FHV-1 alone (4 cats); FHV-1 and aerobes (18 cats); FHV-1 and *Mycoplasma* spp. (0 cats); FHV-1, aerobes and *Mycoplasma* spp. (30 cats), aerobes alone (4 cats), aerobes and *Mycoplasma* spp. (5 cats), and *Mycoplasma* spp. alone (0 cats). *Bordetella bronchiseptica* was isolated from 3 cats; 1 cat was coinfected with other aerobes, FHV-1 and *Mycoplasma* spp., 1 cat was coinfected with FHV-1 and *Mycoplasma* spp., and 1 cat was coinfected with other aerobes only.

In cats of this study, coinfections of FHV-1 with aerobic bacterial or *Mycoplasma* spp. were most common (78.7%). *Bordetella bronchiseptica* infections were uncommon (4.9%). Because FHV-1, aerobic bacteria, and *Mycoplasma* spp. are commonly isolated from normal and clinically ill cats, results of these tests do not correlate to clinical disease in individual cats. Further studies will be required to determine the pathogenic potential of *Mycoplasma* spp. isolated from cats with upper respiratory tract disease.

198 MAGNITUDE OF CRANDELL REESE FELINE KIDNEY CELL LINE ANTIBODY RESPONSES OF CATS ADMINISTERED FVRCP VACCINES.

MR Lappin, RW Sebring, WA Jensen, GR Frank, SV Radecki. From Colorado State University, Fort Collins, CO (Lappin), and Heska Corporation, Fort Collins CO (Sebring, Jensen, Radecki).

Feline herpesvirus 1, calicivirus, and panleukopenia virus for use in feline vaccines (FVRCP) are commonly grown in Crandall-Reese Feline Kidney (CRFK) cells. We previously showed that SQ, but not intranasal (IN) FVRCP vaccines that contain CRFK proteins induce CRFK antibody levels detectable by ELISA. However, only two cats per group were assessed precluding statistical comparisons between groups. The objectives of this study were to determine and compare the magnitude of CRFK antibody responses of cats inoculated with commercially available FVRCP vaccines for IN or SQ administration that are known to contain CRFK proteins.

Fifty unvaccinated kittens were divided into five groups of 10. One of the following was administered to a group of kittens on days 0, 28, and 56: the FVRCP vaccine for IN administration or one of four FVRCP vaccines for SQ administration (P1; P2; P3; P4). Pre-inoculation and day 67 post-inoculation sera