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SESSION X: VETERINARY INFECTIONS & ANAEROBES IN THE FOOD CHAIN

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VETERINARY INFECTIONS & ANAEROBES IN THE FOOD CHAIN

MLST AND MLVA ANALYSIS OF *CLOSTRIDIUM DIFFICILE* GENOTYPES FROM FOOD ANIMALS, FOODS AND HUMANS

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C. difficile associated disease (CDAD) has historically been associated with nosocomial (hospital-acquired) transmission. Community-associated CDAD has increasingly been reported. These data in conjunction with recent findings of *C. difficile* in retail foods has raised concern of *C. difficile* transmission to humans through contaminated foods. While epidemiologic studies investigating the potential of pathogenic food-borne *C. difficile* are difficult to perform, molecular genotyping can provide insight into the genetic relatedness of food, animal and human isolates. A collection of *C. difficile* food, human and animal isolates obtained from a variety of sources and geographic locations were evaluated by multi-locus variable number tandem repeat analysis (MLVA). Multi-locus sequence typing (MLST), *tcdC* genotyping and pulsed-field gel electrophoresis (PFGE) were performed on a subset of isolates for comparison. Genetic relationships were evaluated by minimum spanning tree (MLVA), maximum likelihood analyses (MLST) and dendrograms using the unweighted-pair group method with arithmetic mean (PFGE). In general, food isolates were either identical or highly related by MLVA and formed clusters according to geographic origin. Several food isolates showed identity to animal and human isolates. MLVA provided better discrimination of food, animal and human isolates than MLST or PFGE. Further investigations of *C. difficile* isolates from epidemiologically related food, animal and human sources will be required to demonstrate an etiologic basis for transmission from food to humans.

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PATHOGENESIS OF ENTERIC INFECTIONS BY *CLOSTRIDIUM PERFRINGENS* TYPE A

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Clostridium perfringens type A has long been recognized as a major component of the small intestinal and colonic microbiota. Failure of bacteriologic culture of specimens from the intestines to yield isolates is unusual. This has led many to believe, some quite persistently, that type A is avirulent in the gut: if it is found with equal frequency in the intestines of normal animals, how could it be a pathogen?

Nonetheless, type A has gradually come to be accepted as a disease agent. It is of particular importance as the etiologic agent of poultry necrotic enteritis, a disease that causes about $2 billion annual loss worldwide. Foals and calves develop neonatal hemorrhagic enteritis due to type A infection. *Clostridium perfringens* type A may be the most important uncontrolled cause of enteritis in neonatal pigs. In each of these cases, disease has been reproduced by experimental inoculation.

There is still much to be learned about mechanisms of pathogenesis in type A infections. A recently-described toxin, NetB, is an important contributor to pathogenesis of poultry necrotic enteritis. Alpha toxin (CPA) apparently has a role, as well. In disease of other species, virulence is less well-understood. However, within the general *C. perfringens* type A population, there apparently exist enclaves of strains that have pathogenicity islands, some arranged into pathogenicity archipelagos, which define their virulence for specific animals.

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IDENTIFICATION OF NOVEL PATHOGENICITY LOCI ASSOCIATED WITH AVIAN NECROTIC ENTERITIS-PRODUCING STRAINS OF *CLOSTRIDIUM PERFRINGENS*

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Clostridium perfringens type A causes poultry necrotic enteritis (NE), an enteric disease of considerable economic importance, yet can also exist as part of the normal intestinal microbiota. A recently-discovered pore-forming toxin, NetB, is essential for pathogenesis in most, but not all, NE-causing isolates. This finding suggests that NE-causing strains may also possess other virulence gene(s) not present in commensal type A strains. We used high-throughput sequencing (HTS) technologies to generate draft genome sequences of seven unrelated *C. perfringens* poultry NE isolates, and identified additional novel NE-associated genes by comparison with nine publicly available reference genomes. Thirty-one open reading frames (ORFs) unique to NE-causing strains formed the basis for three highly-conserved NE-associated loci that we designated NELoc-1 (42 kb), NELoc-2 (11.2 kb) and NELoc-3 (5.6 kb). The largest locus (NELoc-1) consisted of *netB* and 36 additional genes, including those predicted to encode two leukocidins, an internalin-like protein and a ricin-domain protein. Pulsed-field gel electrophoresis (PFGE) and Southern blotting demonstrated that our NE-causing strains each carried 3 - 5 large plasmids, and localized NELoc-1 and -3 to distinct plasmids. Sequencing of regions flanking these loci revealed sequence similarity with previously characterized conjugative plasmids of *C. perfringens*. This is the first report that *netB* resides on a large plasmid-encoded pathogenicity locus. Our findings strongly suggest that poultry NE is caused by several novel virulence factors, whose genes are clustered on discrete pathogenicity loci, some of which are plasmid-borne.

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CLOSTRIDIUM PERFRINGENS TYPE A, ENTEROTOXIN, TOXIN GENOTYPES AND RIBOTYPES IN DOGS WITH EPISODIC DIARRHEA

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During six episodes of diarrhea over the last 51 months, we monitored the feces from two dogs for a variety of diagnostic markers indicative of *C. perfringens*. A third dog living with the two affected animals was also studied. Symptoms, usually consistent within an episode, ranged from a mild, watery diarrhea to a mucoid and bloody diarrhea. Occasionally the diarrhea resolved spontaneously and, even within an episode, it was sometimes sporadic. We used quantitative, anaerobic culture on pre-reduced blood agar, with and without prior ethanol shock for the selection of spores. An EIA was used to detect *C. perfringens* enterotoxin (CpE, encoded by the *cpe* gene) in feces and broth cultures. We used PCR to identify, genotype and ribotype *C. perfringens* isolates. It was often necessary to work on multiple isolates from a single sample, representing presumptive isolates with different hemolytic patterns and colony types. This was because individual samples often contained several distinct strains, some potentially enteropathogenic (i.e. *cpe*$^+$), some clearly not (*cpe*$^-$). Five of the six episodes of diarrhea were associated with the presence of fecal CpE and strains of *C. perfringens* Type A carrying *cpe* and able to express the gene *in vitro* coupled with the absence of other known pathogens. Only diarrheic stools were positive for CpE. No formed stool was positive in the EIA. CpE was only present when *cpe*$^+$ isolates were seen. The reverse was not the case and in several instances *cpe*$^+$ isolates were recovered from dogs without fecal CpE and without symptoms. Although sometimes completely absent during health, *C. perfringens* spore and total counts were elevated during CpE diarrhea and only slightly less so in some normal stools, suggesting that culture, and the microscopic scrutiny of Gram stained smears for clostridia and spores may be unreliable features on which to base a diagnosis. Furthermore, PCR for *cpe* may not provide any greater diagnostic utility since *cpe*+ isolates were common in both health and disease. The EIA was therefore the only assay, by itself, reliable for the diagnosis of CpE diarrhea in dogs. Treatment with metronidazole (25 mg/kg twice daily) rapidly led to the loss of symptoms and on most occasions the parallel elimination of both CpE and *cpe*$^+$ *C. perfringens* from the diarrheic dogs.

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ANAEROBIC AND AEROBIC BACTERIA IN SALIVA OF KOMODO DRAGONS *(VARANUS KOMODOENSIS)*

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Background: The popular myth that Komodo dragons' pathogenic bacterial flora causes septicemia and death in their prey has recently been disproven by Fry *et al* (PNAS 2009), who found compound mandibular venom glands with ducts opening between their successive serrated pleurodont teeth. The venom has characteristics similar to that of snake venoms, causing coagulopathy, hypotension, hemorrhage, and shock. The speculation that bacteria are delivered through copious quantities of bloody saliva was also questioned since a variety of mildly virulent organisms, but no single virulent species, have been identified in the few wild or captive dragons previously cultured. Anaerobic bacterial cultures have not been previously reported.
Materials and Methods: We obtained saliva samples from one male and one female dragon housed at the Los Angeles Zoo. Samples were collected by zoo personnel by pipette from dragon drool, inoculated into anaerobic transport media and brought to the lab within one day. The samples were placed into the anaerobic chamber for inoculation onto a series of selective and non-selective agars for anaerobic incubation, and removed from the chamber to inoculate media for aerobic organisms. After incubation at 37°C, the various colony types were subcultured for purity and identified using gram stain, API20E, RapID Strep, Rapid ANA, and other spot tests. Strains with presumptive identifications were further identified using 16S RNA gene sequencing.
Results: The male dragon had 8 aerobic and 3 anaerobic species while the female had 9 and 5 respectively. Both dragons grew 3+ - 4+ enteric bacilli including *E. coli*, *Klebsiella oxytoca*, *Enterobacter* sp. and *Providencia rettgeri*. Both had heavy growth of *Enterococcus faecalis*, and the female also had *Streptococcus dysgalactiae.* Both had coagulase-negative *Staphylococcus* spp. including *S. sciuri,* and a variety of gram-positive rods including *Bacillus cereus-thuringiensis, Rothia* sp. and *Corynebacterium* sp. The female dragon grew *Fusobacterium varium,* two other gram-negative rods resembling *Bacteroides* and *Parabacteroides* spp., *Clostridium perfringens* and *C. sardiniensis.* The male grew *Bacteroides fragilis*, *Clostridium sordellii,* and a swarming *Clostridium* species.
Conclusion: The oral flora of the Komodo dragon is much more diverse than previously reported. No *Pasteurella* species were found in our cultures. It seems likely that their flora represent the gut flora of their last meal, which at the LA Zoo included frozen then thawed entire rats and quail.

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SUSCEPTIBILITY OF HAMSTERS TO EPIDEMIC AND HISTORIC BI/NAP1/027 *CLOSTRIDIUM DIFFICILE* INFECTION DURING DAILY ADMINISTRATION OF FLUOROQUINOLONE ANTIBIOTICS

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The recent epidemic of *Clostridium difficile* infection (CDI) has been attributed largely to the REA strain group BI (aka NAP1/027). Current isolates of the BI group differ from historic non-epidemic BI strains in that they have developed resistance to the newer fluoroquinolone (FQ), moxifloxacin. The acquisition of moxifloxacin resistance has been associated with the rise in frequency of CDI caused by these isolates. In order to determine the effect of FQ resistance on CDI we compared colonization and mortality in hamsters challenged with a historic (BI1 – susceptible to moxifloxacin) and a recent epidemic (BI17 – resistant to moxifloxacin) strain of CD during continuous administration of 3 different FQs.

Groups of 6 hamsters were treated with a 5 day course of ciprofloxacin, (20mg/kg) levofloxacin, (50mg/kg) or moxifloxacin (20mg/kg) given orally once per day. Each hamster was then challenged with $1x10^4$ cfu of either BI1 or BI17 on day 3 or day 5.

Colonization efficiency (CE) following moxifloxacin (92%) was significantly greater than following levofloxacin (50%, $p<0.003$) or ciprofloxacin (42%, $p<0.0005$) for both strains combined. The CE of BI17 was higher than BI1 for ciprofloxacin (67% vs 17%, $p=0.04$) and levofloxacin (83% vs 17%, $p=0.003$), but not moxifloxacin (100% and 83%, $p=0.48$) administration. BI17 also showed a shorter time from inoculation to death than BI1 following moxifloxacin administration (1.8 days vs 3.9 days, $p= 0.000006$). Moxifloxacin shortened the time from inoculation to death compared to ciprofloxacin in hamsters challenged with BI17 (1.8 days vs 4.0 days, $p= 0.009$) but not levofloxacin (1.8 days vs 2.0 days, p= NS). Ciprofloxacin, levofloxacin and moxifloxacin were assayed in groups of 5 hamsters and fecal levels were detected in all (ranging from 11.2-806.1, 20.8-470.3, and 13.4-63.9 µg/g stool respectively).

These data suggest higher CDI frequency and severity with epidemic BI17 than with BI1, but also show increased CDI rates in association with the newer FQ, moxifloxacin.

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ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ANIMAL ISOLATES OF *CLOSTRIDIUM DIFFICILE* BY BROTH MICRODILUTION

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The minimum inhibitory concentration (MIC) of 30 antimicrobial agents was determined by broth microdilution method for 31 isolates of *Clostridium difficile* from pigs (21), calves (5), dogs (4) and a horse (1).

The method was performed on commercially available broth micro-dilution plates for monitoring resistance of anaerobic and Gram-positive bacteria (Trek Sensititre Anaerobe MIC Plate and Gram-Positive MIC Plate). The antimicrobial agents tested were ampicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, cefotetan, cefoxitin, chloramphenicol, ciprofloxacin clindamycin, daptomycin, erythromycin, gentamicin, imipenem, levofloxacin, linezolid, meropenem, metronidazole, mezlocillin, moxifloxacin, nitrofurantoin, oxacillin, penicillin, piperacillin, piperacillin/tazobactam, quinupristin/dalfopristin, rifampin, streptomycin, tetracycline, tigecycline, trimethoprim sulfamethoxazole and vancomycin.

All isolates had low MICs for metronidazole (≤0.5 µg/mL-1 µg/mL) and vancomycin (≤0.25-0.5 µg/mL). For the fluorquinolones, the $MICs_{90}$ were determined for levofloxacin (4 µg/mL), moxifloxacin (2 µg/mL) and ciprofloxacin (>2 µg/mL). For levofloxacin and moxifloxacin, MICs of three strains were >4 µg/mL. For the cephalosporins, the MIC_{90} for cefoxitin was >32µg/mL and for cefotetan 16 µg/mL. MICs for clindamycin were distributed within whole tested range (≤0.25->8 µg/mL, MIC_{90} 8 µg/mL). MICs for erythromycin also varied (≤0.25->4 µg/mL, MIC_{90} 1 µg/mL). MICs for tetracycline showed bimodal distribution: 58% of isolates had MIC ≤0.25 µg/mL and 42% of isolates had MIC 4-8 µg/mL. For the penicillins, the $MICs_{90}$ were determined for penicillin (2 µg/mL), piperacillin (8 µg/mL), piperacillin/tazobactam (8/4 µg/mL), oxacillin (4 µg/mL), ampicillin (1 µg/mL) and amoxicillin/clavulanic acid (≤0.5/0.25% µg/mL). For the carbapenems, MIC_{90} for imipenem and meropenem was 8 and 1 µg/ml, respectively. For rifampin, all isolates had low MICs (MIC_{50} and MIC_{90} ≤0.5 µg/mL), except one strain isolated from a dog (>4 µg/ml).

We found the broth microdilution method convenient to perform, reproducible and MIC endpoints were generally clearly determined. However, in the case of some antimicrobial agents, the dilution range was too narrow to identify resistant strains.

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DIVERSITY ANALYSIS OF METHANOGENS PRESENT IN MURRAH BUFFALOES BASED ON 16S RIBOPRINTING

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One of the major concerns of the world today is Global warming and methane (CH_4) is a one of the most potent green house gases (GHG), having almost 21% more global warming potential than CO_2. Almost 40% of the methane emission in the environment is from ruminant livestock by the process of enteric fermentation. In the present study an effort has been made to decipher the diversity of methanogens by using 16S riboprinting approach. Rumen digesta samples were obtained after manual mixing of rumen contents from five rumen fistulated mature buffaloes (*Bubalus bubalis*). Total genomic DNA was isolated by using Bacterial DNA isolation kit (Fermentas, USA). Genomic DNA was amplified by using methanogen specific primers MET 86F and MET 1340R (Wright et al., 2003). The amplified product was purified and subject to cloning using STRATACLONE Blunt End cloning kit (Stratagene, USA). A total no. of 110 positive clones were screened on the basis of blue/ white screening. Plasmids from randomly selected transformants containing appropriately sized inserts were isolated and PCR was made again to reconfirm the presence of appropriately sized insert in them. PCR products were then extracted from gel and were subjected to ARDRA analysis by using HaeIII restriction enzyme. Restriction digestion products were then run on the 2% agarose gel to analyze different patterns. A total of 12 different patterns (phylotypes) were identified based on restriction digestion analysis confirming the presence of at least 12 different strains of methanogens present in Murrah buffaloes (*Bubalus bubalis).*

Reference:
Wright.A-DG, Pimm. C. *Improved strategy for presumptive identification of methanogens using 16S riboprinting. J. Microbiol. Methods. 2003 ; 55* **:** 337–349

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EVALUATION OF *BACTEROIDES VULGATUS* AND *BACTEROIDES THETAIOTAOMICRON* INTERACTION EFFECTS ON MICE PERITONEAL MACROPHAGES

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The anaerobic bacteria compose the bacterial populations of human microflora. Such bacteria eventually can act as ethyological agents of infectious process when alterations occurs in mucous barrier for example. The *Bacteroides* species are strict anaerobes in Gram negative bacilli form, bile resistant and non spore forming. In terms of virulence, *B. fragilis* are the most representative, since it is more isolated from clinical specimens and because of its resistance profile to antimicrobial agents. However, other species like *B. vulgatus* and *B. thetaiotaomicron* are also capable to establish an infection. Inner microorganism properties such as adhesion, multiplication, toxin production and other virulence factors contribute to the pathogenic behavior. It has been already described that *B. fragilis* is able to escape host immune defenses through alteration of mice peritoneal macrophage microbicide answer, interfering with nitric oxide (NO) production, disturbing actin arrangement and causing damages on macrophages surface. So, the aim of this study was to evaluate *B. vulgatus* and *B. thetaiotaomicron* interaction with mice peritoneal macrophages (MØ) in order to compare with *B. fragilis* results. To reach this purpose scanning electron microscopy (SEM) and NO production analysis were performed after two hour interaction essays. The preliminary results with SEM showed that both "non-fragilis" species can cause damages on MØ, including images suggesting actin filaments extrusion, and can modify NO production on such phagocytes. Such data could suggest a common anaerobic bacteria mechanism to escape from host immune system but more studies are needed to confirm these findings.

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PRESENCE OF *CLOSTRIDIUM DIFFICILE* IN POULTRY AND PHEASANT FARMS

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Young animals with or without disease signs are an important host for *Clostridium difficile*. This study focused on the presence of *C. difficile* in young animals on different poultry and pheasant farms and in farm environment. Altogether 152 samples from poultry from two different farms and 41 samples from pheasants, from a single farm were collected. Farm environment included water from drinking dispensers (poultry, n=4; pheasants, n=2) and litter (poultry farm, n=8). After five days of enrichment and alcohol shock *C. difficile* was isolated on selective agar plates. Isolates were characterized by toxinotyping and PCR ribotyping. Sixty one out of 152 (40,1%) poultry samples and 24 out of 41 (58,5%) pheasant samples yielded *C. difficile* after enrichment. Altogether 162 *C. difficile* isolates were obtained. The proportion of *C. difficile* positive samples (26,8 %) collected at broiler farm was lower than the proportion of *C. difficile* positive samples collected at laying hens replacement farm (44,3 %). Young animals are more likely to be colonized with *C. difficile* than older as isolation rate in a period of 11 weeks of age dropped from 80,0 % to 20,2 % in poultry and from 100,0 % in pheasants sampled at day 3 of age to 30,0 % at day 24 of age. In both, broilers and replacement laying hens, as once already reported, high diversity of PCR ribotypes (16 different PCR ribotypes) was found. In young pheasants a single PCR ribotype (type 131) prevailed, only one out of 64 isolates was of different PCR ribotype. Farm environment could be source of infection for animals as samples of litter and water in small water dispensers were *C. difficile* positive and the same types of *C. difficile* as in animals were found. A subgroup of *C. difficile* positive (n=5) and negative (n=5) poultry stool samples was also analyzed by employing denaturing high performance liquid chromatography (DHPLC) using the WAVE Microbial Analysis System and differences in the composition of fecal flora between *C. difficile* positive and negative samples were detected.

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THE ROLE OF THE GUT MICROBIOTA IN A MURINE MODEL OF *CLOSTRIDIUM DIFFICILE* INFECTION

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Purpose*: Clostridium difficile* is a pathogen that causes nosocomial antibiotic-associated diarrhea and colitis. Employing a recently described murine model of *C. difficile* infection (CDI) we sought to determine the relationship between antibiotic administration, the indigenous GI tract microbiota and *C. difficile* colonization and disease.
Methods: WT C57BL/6 mice were treated for 3 days with a combination of five antibiotics (Abx) consisting of kanamycin, gentamicin, vancomycin, colistin and metronidazole in drinking water. The animals were returned to sterile drinking and two days later received a single dose of clindamycin. One day after receiving clindamycin, the animals were challenged with *C. difficile* (VP1 10463) via oral gavage. Additional groups of animals remained untreated (control), received Abx alone, clindamycin alone, Abx and clindamycin, or clindamycin and *C. difficile*. 16S rRNA-encoding gene libraries and quantitative PCR were used to monitor changes in the community structure after antibiotic administration and *C. difficile* challenge.
Results: A 20-fold reduction in total bacterial load was observed after Abx treatment; however, no effect was observed after treatment with both Abx and clindamycin. Mice that were treated with both Abx and clindamycin prior to challenge with *C. difficile* were readily colonized and remained so for the duration of the study while animals in the other groups did not become stably colonized. Two responses were observed in colonized mice. 58% (7/12) lost > 20% body weight and were moribund between 2-4 days post infection while the remaining 42% (5/12) did not lose significant body weight (well). Moribund animals had a histologic appearance of severe colitis with an active inflammatory infiltrate, epithelial damage and marked edema. The community structure of the gut microbiota was markedly shifted in animals treated with both Abx and clindamycin. Moribund animals were found to have a similar community structure with an abundance of Gamma Proteobacteria. Interestingly, the gut microbial community in "well" mice was more similar to that seen in untreated mice. Diversity was lowest in all moribund mice compared to those that were "well" and untreated.
Conclusion: Antibiotic administration causes major shifts in the community structure and composition of the GI microbiota resulting in a loss of colonization resistance to *C. difficile*. In the animals that develop severe colitis following colonization the gut microbiota is characterized by a predominance of Gamma Proteobacteria and decreased overall diversity.

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OUTER MEMBRANE PROTEINS OF *FUSOBACTERIUM NECROPHORUM* SUBSP. *NECROPHORUM* AND SUBSP. *FUNDULIFORME* MEDIATE ATTACHMENT TO BOVINE ENDOTHELIAL CELLS

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Fusobacterium necrophorum, a gram negative and rod-shaped anaerobe, is an important bovine pathogen that causes rumenitis-liver abscess complex, foot rot, and necrotic laryngitis. Two subspecies are recognized, subsp. *necrophorum* and subsp. *funduliforme.* In cattle, subsp. *necrophorum* is more frequently encountered in infections than the subsp. *funduliforme*. Outer membrane proteins (OMP) of many gram negative bacteria facilitate bacterial attachment to eukaryotic host cells, an initial step in the pathogenesis. The objective of this study was to evaluate the role of OMP of subsp. *necrophorum* and subsp. *funduliforme* of cattle origin in mediating attachment to bovine endothelial cells. Outer membrane proteins from both subspecies were extracted and polyclonal antisera to OMP were raised in rabbits. Analyses by SDS-PAGE revealed four unique bands (19, 28, 35 and 40 kDa) in subsp. *necrophorum*, of which three (19, 35 and 40 kDa) were immunodominant in western blot analyses with rabbit polyclonal antisera or sera from cattle with liver abscesses at slaughter. Bovine adrenal capillary endothelial (EJG) cells, grown in Eagle's minimum essential medium, were used in attachment assays (100:1 ratio of bacterial and endothelial cells). The subsp. *necrophorum* had higher binding affinity for endothelial cells compared to the subsp. *funduliforme*. When bacterial cells were pretreated with polyclonal antiserum (1:100 dilution) raised against OMP of subsp. *necrophorum* or subsp. *funduliforme,* there was a subspecies specific inhibition in the attachment of *F. necrophorum.* The attachment of subsp. *necrophorum* was reduced by 79% with antiserum raised against OMP of subsp. *necrophorum* and the attachment of subsp. *funduliforme* was reduced by 34% with antiserum raised against subsp. *funduliforme.* There was also a significant decrease in the attachment of *F. necrophorum* when endothelial cells were preincubated with OMP. The results suggest the importance of OMP in mediating attachment of *F. necrophorum* to bovine endothelial cells.

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COMPARISON OF THE PREVALENCE AND GENOTYPIC CHARACTERISTICS OF *CLOSTRIDIUM DIFFICILE* IN A CLOSED AND INTEGRATED HUMAN AND SWINE POPULATION IN TEXAS

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The potential for *C. difficile* to be a food borne pathogen is an issue of current debate. One of the possible sources is thought to be swine. We have found the prevalence of *C. difficile* in groups of late production swine to be low (2.7%) and this might suggest a low food safety risk. The objective of this study was to compare the prevalence and genotypic characteristics of *C. difficile* in a closed population in Texas consisting of both human and swine populations from 2004 to 2006 in order to investigate the possible food safety and occupational risks associated with swine and *C. difficile*. Isolation of *C. difficile* was performed utilizing an enrichment technique and restrictive media. PCR was used to test for the presence of the toxin A and B genes, the tcdC gene deletion, and the binary toxin gene. Genotypic characteristics were compared using PCR toxinotyping and PFGE. We tested 2,292 aggregated human wastewater samples from 2004 to 2006 and found 271 (11.8%) to be positive for *C. difficile*. The prevalence of *C. difficile* in worker (12.0%) and non-worker group cohorts (11.6%) did not differ significantly (p=0.81). The majority (84.5%) of the wastewater isolates belong to Toxinotype V. PFGE showed 14 different patterns but there were two dominant patterns. We have found that the majority of our swine isolates also belong to Toxinotype V and the same two dominant PFGE patterns. The similarity in prevalence between swine workers and non-workers show a low occupational hazard of working with swine and *C. difficile* infection. We have found that there is a decreased prevalence of *C. difficile* in late production groups in swine; however, the isolates derived from human wastewater appear to be of a very similar Toxinotype and PFGE pattern to those found in swine.

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SEQUENCE AND PROTEOMIC ANALYSIS OF A TYPE A *CLOSTRIDIUM PERFRINGENS* ISOLATE FROM BOVINE HAEMORRHAGIC ABOMASITIS

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Clostridium perfringens is an anaerobic bacterium found ubiquitously in the soil as well as in the gastrointestinal tract of animals, including humans. While usually a commensal inhabitant, certain factors may lead to the rapid proliferation and secretion of toxins by *C. perfringens* that result in a wide range of pathologies. Type A isolates are sometimes associated with an often fatal haemorrhagic abomasitis of calves. A two-pronged approach incorporating genomics and proteomics is being used to understand the characteristics of *C. perfringens* associated with this disease, in particular to identify and characterize any novel toxin(s) or their genes. Using an isolate of Type A *C. perfringens* obtained from a case of haemorrhagic abomasitis in a calf (F262), whole genome sequencing was carried out using the 454 sequencing platform by Roche. Software tools provided by The McGill University and Génome Québec Innovation Centre, as well as a selection of freeware options, facilitated assembly of the contigs using a mapping approach and some limited *de novo* assembly. The assembly process of the genome will be aided by optical mapping. Annotation of the partially assembled genome will be carried out by NCBI and comparisons made to previously sequenced genomes to identify similarities. Apart from this genomics-based approach, a proteomic avenue is being used. Protein bands separated by SDS-PAGE from the supernatant of a culture of *C. perfringens* F262 are being identified by mass spectrometry; these amino acid sequences will be cross referenced with the genome sequence to identify the corresponding genes. Results from both perspectives will be presented. The synergistic approach of genomic and proteomic platforms is a useful way to understand the basis of haemorrhagic abomasitis of calves.