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SESSION XIV—VETERINARY INFECTIONS

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VETERINARY INFECTIONS

IS *CLOSTRIDIUM DIFFICILE* IN THE FOOD CHAIN?

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Clostridium difficile (CD) is the main cause of antibiotic-associated diarrhea in hospitalized patients, and there is an increasing number of reports of community-onset disease. CD strains from humans are often identical to those causing typhlocolitis in neonatal pigs and diarrhea in other animals produced for food. We investigated retail meats as a source for CD acquisition by humans.

Retail meats and meat products purchased at retail were examined by selective culture. Isolates were characterized by PCR ribotyping, pulsed-field gel electrophoresis (PFGE), and toxinotyping. Binary toxin gene *cdtB* and deletions in *tcdC* were detected by PCR. All isolates were examined for susceptibility to fluoroquinolones and clindamycin. Results were compared to a database of CD strains from human and animal sources.

Toxigenic CD was isolated from 41.1% of retail meats intended for human consumption. Twenty-seven percent of these strains were ribotype 027 and either NAP1 (from braunschweiger, beef summer sausage, and ground beef) or NAP1-related (ground beef, ground pork, pork chorizo, and braunschweiger). Most isolates from foods (73%) were ribotype 078/TT V and either NAP7 (95.6%) or NAP 8 (7.4%). NAP1 meat isolates were 88.9 - 100% related to a NAP1 human isolate. NAP1-related isolates from different meat types were indistinguishable from each other, but only 78% related to NAP1. NAP7 isolates from meats were > 90% related to a NAP7 human isolate, and those identified as NAP8 were indistinguishable from a human NAP8. NAP1 isolates were resistant to fluoroquinolones, while NAP1-related isolates were susceptible; all ribotype 027 strains were clindamycin resistant. Ribotype 078 isolates were susceptible to fluoroquinolones, but only one was susceptible to clindamycin. Ribotype 078/TT V strains are the predominant CD population isolated from diseased pigs and calves in the US, and are increasingly isolated from humans with community-associated disease. These data suggest that domestic animals, by way of retail meats, may be a source of *C. difficile* for human infection.

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ANAEROBIC INFECTIONS IN MARINE MAMMALS

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Marine mammals are charismatic and important natural resources who receive federal protection through the Marine Mammal Protection Act. Sea otters and sea lions inhabit the near-coastal regions, are impacted to a number of anthropogenic activities such as boating, fishing, and both chemical and biological pollution, and are considered to be sentinels of ecosystem health. The current retrospective study was undertaken to determine the prevalence of anaerobic infections in coastal marine mammals to better inform the clinician treating stranded animals and to monitor for fecal pathogen pollution. Microbiology records with the request for anaerobic culture from sea otter and sea lion samples, excluding feces and intestine, submitted to the Microbiology Laboratory at the Vet. Med. Teaching Hospital at the University of California, Davis from 1990 to 2008 were reviewed.

One or more obligate anaerobic bacterial species were isolated from 30% of sea otters. A total of 376 sites were cultured anaerobically; 16% were positive. Lesions or tissues most commonly yielding anaerobes included abdominal fluid, abscesses, lymph nodes, and wounds. The most frequent bacterial genera identified were *Fusobacterium, Clostridium*, and *Peptostreptococcus*. The majority of sites with anaerobes in otters also contained aerobic or facultative anaerobic bacteria most commonly *Escherichia coli, Streptococcus* spp., *Arcanobacterium* spp., *Vibrio* spp., and *Shewanella putrefacians*.

Approximately 31% of sea lions had 1 or more anaerobes isolated. As for otters, abscesses most commonly contained anaerobes. All sites had either obligate aerobes or facultative anaerobes present. *E. coli* was the most commonly isolated facultative anaerobe.

The bacterial genera isolated from sea otters and sea lions are similar to those seen in domestic mammals. The locations of anaerobic infections in marine mammals were comparable to those seen in domestic animals. One caveat of these findings is that the majority of samples came from post-mortem examination. Sampling of tissues for anaerobic culture can be meaningful in sea otters and sea lions both ante- and post-mortem. Knowledge of the conditions or sites where anaerobic infections are likely will help to guide empirical antimicrobial therapy of these animals.

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CLOSTRIDIUM *DIFFICILE* IN ANIMALS: HOW MANY TYPES ARE THERE?

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Clostridium difficile strains can be further differentiated by several molecular methods. Mostly used at the time are pulse field electrophoresis (PFGE), restriction enzyme analysis (REA), and PCR ribotyping. Additional method for differentiation of *C. difficile* strains is toxinotyping based on the changes in region coding for two main virulence factors, toxin A (TcdA) and toxin B (TcdB). Toxinotyping is less discriminative as other typing systems as currently only 28 toxinotypes are described in comparison to >100 types for each of other methods mentioned. However, toxinotyping is a method with good interlaboratory comparability and variant toxinotypes (strains with changes in toxin coding region) correlate well with other molecular typing methods.

The majority of studies on the animal isolates have used PCR ribotyping and sometimes toxinotyping. The diversity of strains found in animals to date is much lower than in humans but it also depends on country or on animal species. Canadian studies report higher number of *C. difficile* types isolated from animals than other countries but are also performed on larger number of farms and/or animals. As for the animal hosts diversity of strains isolated from pigs is lower than described for cattle and poultry.

It is important to note that all types isolated from animals have been previously isolated from humans as well. However, different types are at the time prevalent in both host groups. Interestingly, variant *C. difficile* strains, and in particular strains with binary toxin, are more often associated with animals than with humans. Altogether 7 variant toxinotypes out of 27 known (III, IV, V, VIII, XI, XII, XXII), in addition to toxinotype 0, were to date found in animal hosts.

Countries usually have their own prevalent ribotypes as far as human isolates are concerned, but some types are prevalent in several geographic regions. In the last couple of years, ribotypes 027 or 017 have been emerging epidemic strains in North America, Europe and Asia. Similarly, toxinotype V/ribotype 078 seem to be particularly adapted to animals and is present in different animal hosts (horses, cattle, pigs) all over the world. It can represent up to 22.5% (Canada) or 94% (USA) of cattle isolates or 83% of swine isolates (USA). In humans, ribotype 078 represents from 7% to 17% of isolates, but its prevalence is increasing. Additionally, toxinotype V includes ribotype 078 and 066 and in some countries ribotype 066 but not 078 is found in pigs.

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PREVALENCE OF *CLOSTRIDIUM DIFFICILE* IN AN INTEGRATED SWINE OPERATION

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The objective of this study was to compare the prevalence of *Clostridium difficile* among different age and production groups of swine in a vertically integrated swine operation in Texas in 2006 and to compare our isolates to other animal and human isolates. Isolation of *C. difficile* was performed utilizing an enrichment technique and restrictive media. Preliminary results are based on 131 *C. difficile* isolates arising from 1008 swine fecal samples and pork trim samples (overall prevalence of 13%). The prevalence (number positive/number tested in production type) of *C. difficile* was different between the groups (P<0.001), and was highest among farrowing barn inhabitants (predominantly piglets, but also included lactating sows and influent) at 36.5% (95/260), followed by 8.2% (10/122) for nursery, 6.5% (4/61) for pork products, 3.9% (15/382) for grower-finisher, and 3.8% (7/182) for breeding boars and sows. Isolates were tested for Toxins A and B using commercially available toxin test kits. Of the 131 isolates, 89/103 were positive for toxin A (28 not tested) and 127/131 were positive for toxins A and B. PCR was used to determine toxinotypes, ribotypes, and binary toxin. All 131 isolates harbored the tcdC gene deletion, typical of hyper-virulent toxin producing strains. Isolates were tested for sensitivity to 11 antibiotics. All 131 isolates were resistant to cefoxitin, ciprofloxacin, and imipenem, whereas all were sensitive to metronidazole, piperacillin/tazobactam, amoxicillin/clavulanic acid, and vancomycin. The majority of isolates were resistant to clindamycin; resistant or intermediate to ampicillin; and sensitive to tetracycline and chloramphenicol. There was an increased (P<0.001) number of isolates for the timeframe of Sept.-Feb. compared to Mar.-Aug. PCR and pulsed-field gel electrophoresis techniques will be used to make genomic comparisons of our isolates to those in the CDC database. In this study, we found *C. difficile* most commonly originated among farrowing barn production types (primarily piglets) and not in grower/finisher production. Relatively low prevalence in late production suggests a low food safety risk; however, the isolates of *C. difficile* in our study are considered more virulent because of the *tcdC* gene deletion, and similar isolates have been linked to outbreaks of *C. difficile* in hospitals of North America and Europe.

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NEUTROPHILE CHEMOTAXIS INDUCTION AND IL-1β, IL-8, TNF-α, IL-11 AND IL-17 PRODUCTION BY ORAL *PORPHYROMONAS* SPP. AND *FUSOBACTERIUM* SPP. FROM DOGS

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Neutrophile chemotaxis induction and cytokine production by oral *Porphyromonas* spp. and *Fusobacterium* spp was determined. Thirteen *Porphyromonas gulae,* 2 *P. macacae*, 2 *P. crevioricanis*, 1 *P. circumdentaria*, 1 *P. gingivicanis*, 1 *P. catoniae,* 12 *F. canifelinum* and 8 *F. nucleatum* were identified. The chemotactic assay in vitro and neutrophil activation to induce cytokine production were performed. All bacterial species were able to induce neutrophile chemotactic activity. The levels of IL-1β, IL-8, TNF-α and IL-11 production were significantly higher when stimulated cells were used. IL-11 was not produced when stimulated by *Fusobacterium* species and no strain caused IL-17 production. Our results demonstrated the ability of different periodontal bacteria from dogs to attract a large number of human neutrophiles and, when in contact with human neutrophiles, the ability to stimulate different cytokines.

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OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *PORPHYROMONAS GULAE, PORPHYROMONAS MACACAE, FUSOBACTERIUM NUCLEATUM* AND *FUSOBACTERIUM CANIFELINUM* ISOLATED FROM DOGS WITH AND WITHOUT PERIODONTITIS

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In this study, the occurrence of *Porphyromonas gulae, Porphyromonas macacae, Fusobacterium nucleatum* and *Fusobacterium canifelinum* isolated from dogs and their antimicrobial susceptibility were determined. Subgingival biofilms were collected from fifty dogs with and fifty without periodontitis. Fifty-three *P. gulae*, 8 *P. macacae*, 38 *F. nucleatum* and 26 *F. canifelinum* were isolated. Hemolytic activity, serum resistance, hemagglutination and hemagglutination inhibition assays, and antimicrobial susceptibility testing were performed. All the evaluated bacteria showed variable phenotypic characteristics. Different resistance rates to clarithromycin, erythromycin and metronidazole were observed. The role of *P. gulae, P. macacae, F. nucleatum* and *F. canifelinum* in periodontal disease of household pets needs to be defined. Our results suggest that the presence of these species in the periodontal sites may play an important role in the establishment and progression of periodontal disease.

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THE ROLE OF *DICHELOBACTER NODOSUS* AND *FUSOBACTERIUM NECROPHORUM* IN FOOTROT IN NEW ZEALAND

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Statement of purpose: To elucidate the role of *Fusobacterium necrophorum* and *Dichelobacter nodosus* in ovine footrot.

Methods and Results: Ovine footrot is a debilitating disease of sheep resulting in lameness, production loss, and suffering. The disease process is the result of a highly complex and poorly understood interaction between multiple anaerobic species, environmental factors, and the host. To understand the basic bacteriology of the pathogens in the 'wild', a survey of healthy and symptomatic footrot infected sheep was conducted with farmers. Species specific PCR's were developed and optimised to test swabs for the presence of the primary pathogens assumed to be associated with footrot; *Dichelobacter nodosus* (*D. nodosus*) and *Fusobacterium necrophorum* (*F. necrophorum*). Of the 45 swabs taken from symptomatic footrot infected sheep, 17 were both positive for both *F. necrophorum* and *D. nodosus*, 4 were positive for *F. necrophorum* only, and 2 for *D. nodosus* only. Of the 51 swabs received from healthy asymptomatic sheep, 1 was positive for *F. necrophorum*. This shows that *F. necrophorum* and *D. nodosus* are statistically significantly linked to footrot and to each other in the field under a normal pastoral farming system.

To further understand *F. necrophorum* role in footrot, SSCP gels were combined with sequencing, this resulted in sequence data of individual PCR products amplified from a variety of disease sources. Two preliminary results were immediately apparent; the portion of the leukotoxin gene targeted by the *F. necrophorum* specific PCR was far more varied than expected; symptomatic footrot infected sheep appeared to only carry a single variant. This study is still in its preliminary stages with only a small number of samples processed so far.

Conclusions: *F. necrophorum* and *D. nodosus* are closely associated with footrot in sheep, either as causative agents or opportunistic colonisers of footrot lesions. The leukotoxin of *F. necrophorum* may be highly varied and a single variant of *F. necrophorum* leukotoxin might be present on footrot infected sheep in New Zealand.

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PHYLOGENETIC ANALYSIS OF *PORPHYROMONAS* SPECIES ISOLATED FROM THE ORAL CAVITY OF AUSTRALIAN MARSUPIALS

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We determined the phylogenetic relationships of a collection of catalase positive black-pigmented anaerobic bacteria belonging to the Genus *Porphyromonas* isolated from the oral cavity of marsupials. *Porphyromonas* species have been isolated from soft tissue infections and gingival margins of cats; from the subgingival plaque of dogs with naturally-occurring periodontitis, and from infected dog and cat bite wounds in humans. Plaque was collected and cultivated from the gingival margins of Brushtail Possums (*Trichosuru vulpecula*), Koalas (*Phascolartctos cinereus*), Eastern Grey Kangaroos (*Macropus giganteus*), a Musky Rat Kangaroo (*Hypsiprymnodon moschatus*), a Red-necked Wallaby (*Macropus rufogriseus*) and Pretty-faced Wallaby (*Macropus parryi*). Black-pigmented isolates were identified as *Porphyromonas gingivalis* were included with nine strains of *Porphyromonas gulae* isolated from North American animals (a bear, wolf, coyote, cat, and a dog species); fourteen strains, previously reported as the animal biotype of *Porphyromonas gingivalis* isolated from Australian cats and dogs, and two *Porphyromonas gingivalis* strains isolated from humans. Porphyromonads isolated from marsupials were compared to the animal species (*Porphyromonas gulae*) and human species (*Porphyromonas gingivalis*) using 16S rRNA gene sequencing. The results of the phylogenetic analysis indicated that three distinct groups were identified. Group 1 consisted of *Porphyromonas gingivalis* strains, Group 2 consisted of a number of sub-groups of *Porphyromonas gulae* isolates and Group 3, a genetically distinct group. The North American isolates belonged to *Porphyromonas gulae* clades. The Australian cat and dog isolates also belonged to *Porphyromonas gulae*. However, the marsupial isolates belonged to either *Porphyromonas gulae* or the genetically distinct group which may constitute a new species of *Porphyromonas*. In conclusion, we speculate that the marsupial *Porphyromonas* isolates (group 3) appear to be unique to marsupials and maybe ancestral to both *P. gulae* and *P. gingivalis*. Marsupial *Porphyromonas* isolates appears to be host-adapted whereas *P. gulae* isolates appears non-host specific being isolated from marsupials and carnivores.

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CHARACTERIZATION OF *CLOSTRIDIUM PERFRINGENS* TOXINOTYPES ISOLATED FROM SEA OTTERS AND MUSSELS FROM THE CENTRAL CALIFORNIA COAST

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Mussels have often been used as bioindicators of environmental pollution due to their filter-feeding capacity. Sea otters eat approximately 25% of their body weight each day, including prey items, such as mussels, and are considered nearshore sentinels for coastal water quality and ecosystem health. In order to evaluate the diversity of *C. perfringens* and whether otters may be exposed to fecal pathogens through their prey species, we isolated and toxinotyped *C. perfringens* from: (1) sea otter fecal and/or gastrointestinal samples and (2) mussel samples collected from different areas along the central California coast, including sites deemed at higher or lower risk for fecal pollution based on distance from known sewage outfalls, livestock runoff, or neither of these sources. Sea otter samples and mussel digestive glands were cultured anaerobically on Egg Yolk Agar (EYA), with identification based on morphology on EYA, hemolysis on Brucella blood agar, and CAMP reaction. Confirmed isolates were characterized with a multiplex polymerase chain reaction analysis capable of detecting the exotoxin genes: enterotoxin, alpha, beta, beta 2, epsilon, and iota. The results demonstrated that there is one predominant toxinotype, Type A, found in both mussels and sea otters. Rare otter isolates were Type C or E. The *C. perfringens* enterotoxin gene was detected in 8% and 14% of sea otter and in mussel isolates, respectively. The beta 2 gene could be detected in 2 otter isolates.

Conclusion: Toxinotype A is the most common *C. perfringens* in central California coastal environments, with additional toxinotypes detected rarely in
sea otters and their prey.

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OCCURRENCE OF PERIODONTOPATHIC BACTERIA IN NON-HUMAN PRIMATES WITH DIFFERENT PERIODONTAL CONDITIONS

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In this study, the occurrence of periodontopathic bacteria in 52 capuchin monkeys (*Cebus apella*) was evaluated: 14 monkeys with gingivitis, 13 with periodontitis and 25 healthy. The animals were maintained in the Center for Experimental Procreation of Capuchin Monkey-São Paulo State University-UNESP, Brazil. Subgingival samples were collected with paper points from gingival crevice or periodontal pocket. DNA was obtained and the qualitative and quantitative presence of periodontopathogens was performed by PCR and RT-time PCR. In the monkeys with periodontitis, *Prevotella intermedia, Porphyromonas gingivalis, Fusobacterium nucleatum, Eikenella corrodens* and *Tannerella forsythia* were detected in 61.54%, 30.77%, 76.92%, 76.92%, and 46.15 %, respectively. *Aggregatibacter actinomycetemcomitans* was predominant in gingivitis. *Treponema denticola, Dialister pneumosintes* and *Prevotella nigrescens* were rarely detected. *Porphyromonas gulae* was detected in 30-40% of the samples. Our results show that *C. apella*, a New World monkey, harbors a diverse microbiota which can be associated to periodontitis and or gingivitis.

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PREVALENCE AND DIVERSITY OF *CLOSTRIDIUM DIFFICILE* IN POULTRY, PIGS AND CALVES

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Clostridium difficile is gaining importance as an animal pathogen. Most of the recent data from Canada and the USA describe high isolation rates from pigs and somewhat lower isolation rates from calves. We have reported the isolation of previously unreported animal PCR ribotype (066) of *C. difficile* from piglets from Slovenian farms. Here we describe the further studies of isolation of *C. difficile* from different farm animals.

From the pigs (aged mostly 1 to 10 days) altogether 430 samples were collected and 213 (49.5 %) of them were culture positive. Four different farms were included in the study, and all were sampled more than once. A single PCR-ribotype (066, toxinotype V, A+B+CDT+) prevailed at three farms and represented 78.4 % of all piglet strains. At the fourth farm unrelated (but unidentified), PCR ribotype was present (toxinotype 0, A+B+CDT-).

Calves (<12 weeks) were sampled from more than 10 different farms. In the first sampling interval, *C. difficile* was cultured onto the selective agar directly from the swabs/fecal samples, and only one sample of 56 was positive. During the second sampling interval enrichment was introduced, and 4 of 36 (11.1%) calve samples were positive. Isolates belonged to toxinotypes XI (ribotype 033, A-B-CDT+) and toxinotype 0 (ribotype not determined, A+B+CDT-).

Three different poultry populations were included in the study, one from larger production farm and two from domestic farms keeping up to 20 animals. Fecal samples were collected from the ground, and *C. difficile* was isolated on selective plates after enrichment step. From domestic chickens none (location 1) or 50% (location 2) of fecal samples yielded *C. difficile*. However, large farm was sampled twice and 71.4 % and 95.8% of fecal samples were culture positive. Chicken isolates show marked diversity and belong to two different toxinotypes (0 and IV) and 12 different ribotypes.

Our data suggest that in addition to pigs and calves, poultry can be an important reservoir for *C. difficile*.

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METHANOMIROBIUM PHYLOTYPES ARE THE DOMINANT METHANOGENS IN BUFFALOES (*BUBALUS BUBALIS*) FROM INDIA

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Methane production in ruminants is an integral part of rumen fermentation. During this process 6%-12% of ingested feed energy is wasted. Moreover this methane accumulation in the environment also contributes towards global warming. The main micro-organisms responsible for methane production belongs to the family archaea. These are strict anaerobes grows only at -350 redox potential. Most of the methanogens are unculturable only few strains are reported to grown under anaerobic conditions. In India, the study on rumen methanogens is still lacking. Recent biotechnological approaches open the way to identify more methanogens in detail. The present study was carried out in Indian buffaloes (*Bubalus bubalis*) to see the different methanogens present in rumen using molecular techniques. Clarified rumen fluid was taken from three buffaloes fed on standard diet. Total Genomic DNA was isolated, and PCR amplification was carried out by using archaea specific primers MET86F and MET1340R (Wright *et al*., 2004). PCR product was purified and cloned in to a suitable vector by using TOPO-TA cloning kit (Invitrogen) with ampicillin and blue white screening, and positive clones were selected. (GenElute plasmid miniprep kit (Sigma). The Plasmid DNA was reamplified by PCR by using the same conditions as used previously. Positive clones (n=45) were sequenced bidirectionally and the resulting sequences were align by using CLUSTAL W software. The resulting sequences, analyzed by using BLAST software reveals that 44 the sequences shows maximum proximity with methanomicrobium mobile in different percentages (98%-99.7%), whereas one sequence seems to be novel. This similarity of most of the sequences with methanomicrobium mobile indicates that methanomicrobium mobile is the dominant phylotype among the rumen methanogens in buffaloes in India. Our results are in contrast with one of the studies conducted on western sheep, where methanobrevibacter is the dominant phylotype (Wright *et al*., 2007). However some more studies in this area are needed to confirm the results.

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DETECTION OF METHANOGENIC ARCHAEA IN STORED SWINE MANURE BY DIRECT *mcrA* PCR AND SEQUENCE ANALYSIS

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Storage of swine manure is associated with the production of a variety of odors and emissions which are products of anaerobic metabolism of the indigenous bacteria in the manure. These emissions can pose problems to the health and production efficiency of the animals, as well as the health and comfort of human workers. Consolidated storage of swine manure also leads to production of large quantities of methane, a greenhouse gas that contributes to global warming. Emissions from animal waste account for about 30% of the total U.S. methane emissions from agriculture. In the United States, methane emissions from lagoons and manure storage pits are estimated to be over 40 Tg/year. Methane is a product of the anaerobic metabolism of methanogenic archaea. Little is known about the population of methanogens in stored swine manure, and surprisingly few methanogens have been isolated from this environment. This study was initiated to identify the methanogenic archaea population of stored swine manure using a culture-independent technique targeting a methanogen-specific functional gene, methyl coenzyme-M reductase subunit A (*mcrA*). Methyl coenzyme-M reductase catalyzes the final step of methane production and is ubiquitous in methanogens. Total DNA was isolated from stored swine manure from local swine facilities and used as a template for direct PCR. Amplification with a degenerate primer set flanking a variable region of the *mcrA* gene generated a PCR product between 464 and 491 bp. These fragments were used to generate a *mcrA* clone library and 50 clones were selected at random for sequencing and comparative phylogenetic analysis. A predominant number of clones were from the *Methanobacteriales* and *Methanococcales* orders with similarities to *Methanosphaera* and *Methanobrevibacter* species. Many clones showed little similarity to any identified methanogens suggesting that these sequences represent novel, as yet unidentified methanogenic archaea. This study is the first *mcrA*-targeted analysis of the methanogenic archaea population of stored swine manure. The presence of previously unidentified methanogens suggests a new source of microbial diversity and new and novel archaea. Results from this study will be used to further develop molecular methods to monitor different populations of methanogens in this environment.

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VETERINARY INFECTIONS

PATHOLOGY OF *CLOSTRIDIUM PERFRINGENS* ALPHA AND EPSILON TOXINS IN BOVINE SMALL AND LARGE INTESTINES

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Clostridium perfringens is a sporulated anaerobe and the causative agent of various diseases in animals and man. *Clostridium perfringens* alpha and epsilon toxins are considered to produce enterotoxaemia in small ruminants. Although *C. perfringens* has been associated with enterotoxaemia and /or necrotic enteritis in bovines, the information regarding the physiopathology of alpha and epsilon toxins in the bovine intestine is either scanty or nil. In this study, intestinal loops were performed in the ileum and colon of three one-week-old Holstein calves. Laparotomy was performed in all three calves under anaesthesia and four loops -three cm long-, were performed in the small and large intestines. For both intestines, one loop was inoculated with alpha toxin (300 LD_{50}), one with epsilon (4000 LD_{50}) and the remaining two with vehicle (PBS) which were used as controls. All calves were kept under anaesthesia at room temperature for four hours after which, euthanasia was performed. Tissue samples from all loops were obtained and fixed in 10% buffered formalin and processed for routine histology. A one millimetre sample of each loop was also obtained and fixed in 2% buffered glutaraldehyde and processed for transmission electron microscopy. Mild congestion was the only macroscopic change observed in the loops, though toxin treated loops were more congested than the controls. Mucous accumulation in the gut lumen was prominent in all treated loops, but in epsilon treated ones, the mucous was also haemorrhagic. The histology revealed high number of exfoliated epithelial cells in the lumen of alpha treated loops, and severe haemorrhage was observed in the lamina propria of epsilon treated colonic loops. Despite some necrotic exfoliated enterocytes, no ultraestructural changes were observed in alpha-treated loops, though epsilon-treated loops exhibited opening of the tight junctions in both, small and large intestines. No ultra-structural changes were observed in the controls. These observations indicate that both, alpha and epsilon toxins can alter the intestinal barrier, predominantly in the large intestine in calves and these toxins are potentially pathogenic for this species. These observations also coincide with previous studies performed in rats and mice.